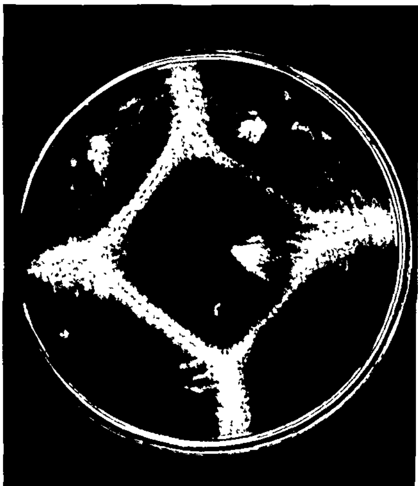
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THE 'BARRAGE' PHENOMENON IN *PODOSPORA ANSERINA*

Simultaneous confrontation of an *S* mycelium with two *S* and two *s* mycelia. A barrage is formed on the contact line between *S* and *s* (courtesy of Rizet, unpublished).

NUCLEO-CYTOPLASMIC RELATIONS IN MICRO-ORGANISMS

THEIR BEARING ON
CELL HEREDITY AND DIFFERENTIATION

BY

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BEING
THE WILLIAM WITHERING
MEMORIAL LECTURES
DELIVERED AT
THE BIRMINGHAM MEDICAL SCHOOL
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'. . . si la science découvre peu à peu l'unité d'un univers accessible à nos procédés d'analyse, c'est par le fait de caractéristiques très générales que la thermodynamique résume en quelques principes. Or, d'après ceux-ci, aucun fonctionnement n'est imaginable s'il n'existe d'abord quelque chose que l'on puisse, même en première et grossière approximation, comparer à une machine, c'est-à-dire une répartition spatiale de matière et d'énergie assez particulière et assez inégale pour que l'énergie utilisable puisse se manifester par un effet. Cette inégale répartition représente l'essentiel d'une structure, dont elle consacre la primauté . . . .

. . . La structure d'un organisme est capable de s'accroître, c'est-à-dire de se modifier tout en restant elle-même; et aussi de se réparer; son état actuel est à la fois un devenir et un résultat; le fonctionnement *tend à la maintenir contre toute cause de perturbation* et d'usure; elle répond pour chaque espèce et pour chaque individu à un type fixe et spécifique qui se reproduit en identité à travers des mécanismes compliqués et divers. Ces quelques remarques confondent avec la question de structure proprement dite un nouveau problème, celui de l'individualité du vivant; et l'on doit conclure que l'organisme est un tout; qu'il ne peut être compris que dans son ensemble, dans la coordination de ses structures et la synergie de ses effectivités; qu'il doit être considéré, en un mot, comme une unité structurale et fonctionnelle.'

(E. FAURÉ-FREMIET, 'Le problème de l'organisation et ses aspects physicochimiques', *Revue Scientifique*, 1943, p. 433.)

PREFACE

'I am a firm believer that without speculation there is no good or original observation'
(DARWIN, Letter to A. R. Wallace, 22 December 1857)

THIS book is a somewhat expanded version of three lectures given in May 1952, at the University of Birmingham, under the auspices of the William Withering Memorial Lectureship.

Invited to give these lectures, I naturally chose to speak on a subject to which I am devoting my own efforts, the genetics of micro-organisms. This new field of research has yielded, in the few years of active exploration, a number of results of interest in many connexions. The facts I have chosen for presentation are those of particular importance in connexion with the problems of cell heredity and differentiation which have determined, to a large extent, my own interest in this field of research. My presentation of the factual material is therefore not exhaustive.

Nor is the speculative part of the book unbiased: it defends a thesis. I have tried to show that the method of Mendelian analysis applied to the classical materials of genetics, while it achieved the amazing progress contained in the theory of the gene, and thus supplied a basis for the understanding of at least one aspect of evolution, has at the same time confined our attention to the nuclear genes and thus driven us into an impasse with respect to the understanding of development. By quoting some genetic studies in micro-organisms, I have tried to show that the cytoplasm is endowed with genetic properties of its own, and that this provides us with a basis for the interpretation of the phenomena of differentiation and development. I do not deny the general occurrence and significance of nuclear (Mendelian) heredity. The bias of my presentation is in the emphasis on the rather

exceptional phenomena of cytoplasmic heredity. This emphasis, as the reader will see, is due to *a priori* reasons suggested by work in non-genetic lines, chiefly experimental embryology, to which most geneticists have in the past paid little attention. I am therefore aware that many of the suggested deductions may not stand the test of time. I know also that very few of my fellow geneticists would subscribe to the views I have expressed, but I am encouraged by the thought that 'we cannot determine the truth of a hypothesis by counting the number of people who believe it' and that 'a hypothesis does not cease to be a hypothesis when a lot of people believe it'.*

My chief purpose in presenting the subject as I did, was to draw attention to some problems which, in my opinion, are of paramount importance. For giving me this opportunity, I am grateful to the sponsors of the William Withering Memorial Lectureship and to the officers of the University of Birmingham.

The three lectures were written before I gave them, and are printed here as they were written. In preparing the manuscript for publication, I have made some additions to the original text, which are inserted in smaller type. Exceptions to this are: a few notes added to the General Discussion, which will be found at the end of the Third Lecture, and some comments 'On the Scarcity of Evidence of Cytoplasmic Heredity and on the Ambiguity of the Concept of Autoreproducing Units' placed in the Addendum, at the end of the book.

The bibliography given at the end of each chapter is not exhaustive. The references have been chosen so as best to guide the reader who would like to acquaint himself more thoroughly with particular subjects. Therefore, the references given are often to general reviews rather than to specialized papers.

* J. H. Woodger, Observations on the present state of embryology (*Symp. Soc. Exp. Biol.* 1948, 2, p. 354).

The majority of the illustrations have either been specially prepared for this book, or redrawn from various sources indicated in the legends. For this excellent work I wish to express my appreciation to Mrs. S. Chevais. I am equally grateful to Dr. G. Rizet who kindly supplied the photographs and diagrams illustrating his magnificent work on *Podospora*, and to Dr. A. Lwoff for supplying the beautiful original drawing of the infraciliature of *Gymnodinioides* (Fig. 1) and a copy of Fig. 2. Lastly, thanks are due to the Editors of *Heredity* and to the Connecticut Academy of Arts and Sciences for the permission to reproduce Figs. 22 and 38, respectively.

B. E.

PARIS

July, 1952

CONTENTS

I

'Inheritance must be looked at as merely a form of growth'
(DARWIN, *Variation in Animals and Plants*)

I. GENERAL INTRODUCTION

THE most decisive step in the development of biology into a scientific discipline was the formulation of the Cell Theory. Its original content is summed up in this sentence of Virchow, the founder of cell pathology: '*Every animal appears as a sum of vital units*, each of which bears in itself the complete characteristics of life.'[33] The emphasis in the quoted sentence is on the cell as a vital unit, and this unit, in Virchow's time, was regarded as the *ultimate* unit of life.

You know that among all the characteristics of life, none of which taken singly permits one to distinguish the living from the non-living,[30] the most astonishing and the most profoundly significant one is the faculty of identical perpetuation, resulting in the permanence of the multitude of living forms.

With the demonstration of cell division, and with the accumulating evidence that a cell can arise only from a pre-existing cell, the fact of identical perpetuation received the beginnings of an explanation.

A series of brilliant discoveries soon came to lay the foundation of the modern science of heredity: the discovery of the fundamental role of the nucleus in the life of the cell, and of the individuality and permanence of its chromosomes; the discovery of the nature of fertilization and notably of the fact that the only link between successive *generations* are single-celled gametes, one of which most often consists of barely more than a nucleus. This led to the notion of genetic determinants and pointed the way to the chromosome theory of heredity. Following the

rediscovery of Mendel's laws of transmission of hereditary traits *in sexual reproduction*, which proved the discreteness of the hereditary material, more refined genetical studies established the complete similarity in the behaviour of groups of the hypothetical genetic determinants (the Mendelian genes) and the visible chromosomes. The gene theory, almost in its present shape, was born: you can see how its development shifted attention, on the one hand, *from the subtle identical perpetuation involved in any sort* of growth and multiplication to that particular form of identical perpetuation which strikes the eye as soon as we turn to sexual reproduction; and, on the other, from the cell as a whole to one of its constituents.

These shifts of attention were, of course, dictated in part by opportunity. Singly, the fact of identical perpetua*tion, in cell division for example*, does not provide proof of the existence of genetic determinants. The demonstration of their existence requires, as you know, the occurrence of genetic variation in the first place, and of the possibility of crossing individuals carrying different forms of the same determinants in the second. Thus, for the purpose of analysis, attention was focused on sexual reproduction.

The demonstration of the existence of genetic determinants was, of course, a tremendous achievement in the analysis of the cell. Taken together with the countless proofs that Mendelian genes control an amazing variety of functions, and, above all, with the fact, so clearly shown by Muller in a paper,[29] in many ways prophetic, written in 1926, that the genes possess the unique, fundamental property of identical reduplication, which would otherwise have had to be ascribed to the cell as a whole, it led to the notion of the nucleus as the governing body of the cell, while the simultaneous absence of evidence for an equally universal cytoplasmic heredity incited geneticists to look at the rest of the cell as a by-product of gene activity. Moreover, the ability of the genes to vary and,

when they vary (mutate), to reproduce themselves in their new form, confers on these cell elements, as Muller has so convincingly pointed out, the properties of the building blocks required by the process of evolution.

Thus, the cell, robbed of its noblest prerogative, was no longer the ultimate unit of life. This title was now conferred on the genes, subcellular elements, of which the cell nucleus contained many thousands and, more precisely, like Noah's ark, two of each kind.

However, the nuclear genes did not remain long the sole possessors of the title. Viruses were soon shown to be endowed with the same marvellous property of identical or *covariant* reproduction. Although viruses were soon obtained in crystalline form, the question was raised whether they were living or not living. The question obviously has no answer, because life cannot be characterized by a single criterion: no more by the criterion of self-reproduction than by any other. As Szent-Györgyi half-jokingly said, under the auspices of this same Lectureship: 'Everyone knows this much Biology—that one rabbit could never reproduce itself, and if life is characterized by self-reproduction, one rabbit could not be called alive at all, and one rabbit is no rabbit, and only two rabbits are one rabbit.'[37]

I am quoting Szent-Györgyi at this point because his remark is a sound protest against judging life by the sole criterion of self-reproduction. But the argument has a flaw, which illustrates what I have mentioned already: the excessive concentration of attention on the self-reproduction involved in sexual transmission of characters from parent to offspring. *The rabbit began by being a rabbit's egg*; the rabbit was gradually evolved in its increasing and exquisite complexity, through the numerous divisions of a single cell. Even if, starting with a microscopic mass of rabbit protoplasm, we had ended up simply with a pound of exactly the same stuff, we would have had to conclude that the material we started with was endowed with the

property of identical reproduction. We could have ascribed this to nuclear genes. *But the development of a rabbit obviously involves much more than making more rabbit protoplasm. It involves both variation of descendants of the initial cell, resulting in a branching lineage of different cell types, and the inheritance of these variations.* The inheritance of the differences between somatic cells, *cell heredity*, is claimed here not only because the mass of each of the differentiated cell types increases during the development and subsequent growth of an individual, but above all because it has been shown by experiments in tissue culture that each cell type can 'breed true' for a practically indefinite time.

Here we have identical perpetuation again, but, in this case, the inherited differences can hardly be ascribed to nuclear genes, for the different cell types which make up a rabbit are all derived from the egg cell by equational mitosis: they must therefore all possess the same genotype. How then can differentiation be explained? To what are the apparently permanent differences between somatic cells due?

Unless development involves a rather unlikely process of orderly and directed gene mutation, the differential must have its seat in the cytoplasm.

The cytoplasm of the egg is indeed heterogeneous, and different cell lines take their origin in portions of cytoplasm of different composition. But it must be emphasized again that, using Medawar's words, 'the mere sharing-out of ancestral cytoplasm among daughter cells and then in turn among their progeny is not a sufficient explanation of cellular inheritance, since the number of claimants to the legacy increases in the extreme case exponentially while the legacy itself is fixed'.[28] *No, if the cytoplasm causes differentiation, it must be endowed with the power of perpetuation of cell-type.*

Consideration of the phenomenon of differentiation thus leads us to a seemingly simple alternative, but the

experimental evidence, at least at first sight, lends no support to either of its terms. On the one hand, the outstanding role of the nucleus in the life of the cell is established beyond doubt, and it is natural to expect it to play a decisive part in cell differentiation. In fact, the earliest theories of differentiation postulated the sorting out of nuclear determinants in the course of development. However, the equational character of nuclear division has been proven by the functional equivalence of the nuclei in the early stages of development, and geneticists know no means of inducing specific gene mutations. This seems to point to the cytoplasm as the seat of the causes of the persistent changes involved in differentiation. But, on the other hand, genetic experiments have revealed cytoplasmic heredity only in exceptional cases. As S. Wright pointed out, the chief objection to the cytoplasmic theory of differentiation 'is that it ascribes enormous importance in cell lineages to a process which is only rarely responsible for differences between germ cells, at least within a species'.[47]

Obviously, what is required is more than deductions from the behaviour of germ cells; what is needed is direct genetic analysis of somatic cells, for the assumed functional equivalence of irreversibly differentiated somatic *cells*, however plausible, is only an hypothesis. Crosses between such cells being impossible, only nuclear transplantation from one somatic cell to another, or grafting of fragments of cytoplasm, could provide the required information; such experiments however will have to await the development of adequate technical devices.* In the meantime, the

* Nuclear transplantation has been *successfully* accomplished in amoebae by Comandon and de Fonbrune[1] and by Lorch and Danielli.[33] A recent paper by Briggs and King[3] gives a first account of successful transplantation of nuclei from frog blastulae cells into enucleated *frog* eggs. Since the nuclei of blastula cells are, according to the authors, almost as small as those of *differentiated* cells of older embryos, it may be hoped that experiments performed *with the* help of the technique of Briggs and King will, in the near future, yield most interesting *information*.

property of identical reproduction. We could have ascribed this to nuclear genes. *But the development of a rabbit obviously involves much more than making more rabbit protoplasm. It involves both variation of descendants of the initial cell, resulting in a branching lineage of different cell types, and the inheritance of these variations.* The inheritance of the differences between somatic cells, *cell heredity*, is claimed here not only because the mass of each of the differentiated cell types increases during the development and subsequent growth of an individual, but above all because it has been shown by experiments in tissue culture that each cell type can 'breed true' for a practically indefinite time.

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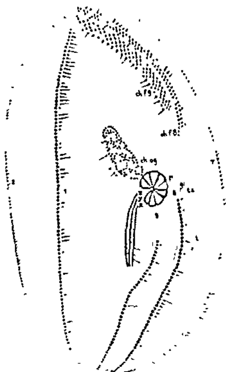


FIG. 1 The system of kinetosomes of the tomite of *Gymnodinioides inkystans* (from Chatton and Lwoff, 1935, see Ref. 2)

closest approximation to the evidence we would like to have is provided by the study of lower forms which propagate by vegetative reproduction and possess no isolated germ line. Studies of some micro-organisms have brought to light some facts of great interest on the respective roles of the cytoplasm and the nucleus, and in the following discussion I intend to present the evidence which appears to me of particular significance in connexion with the problems of cell differentiation and cell heredity. You will see that these studies confirm the view that the cytoplasm, like the genes, is endowed with genetic continuity. The genes are therefore no longer to be regarded as the sole cell-constituents endowed with this property. Moreover, these studies will reveal to us some nucleo-cytoplasmic relations which will permit us to return to a more general consideration of embryonic development. And this, you will see, will lead us to restore to the cell a great part of its lost prestige.

2. VISIBLE CYTOPLASMIC ELEMENTS ENDOWED WITH GENETIC CONTINUITY

Since we are going to be concerned to a large extent with the cytoplasm, let me begin with the following remark. If the cytoplasm has genetic properties of its own, these properties may or may not be linked with particulate elements. The existence of particles endowed with genetic continuity can be suggested by simple observation. The existence of autonomous cytoplasmic properties not correlated with the presence of particulate elements can be suggested only by experiment. The same is true for the rigorous demonstration, *not simply the suggestion, of the* genetic continuity of particulate elements. Since our knowledge of cells in general, and of micro-organisms in particular, was, to begin with, based solely on observation, it is not surprising that first to be discovered was the existence of cytoplasmic particles apparently endowed

with genetic continuity. The experimental proof of this property of some of the particles came later. Genetic experimentation, which is the only means of demonstrating those genetic properties of the cytoplasm that are not linked to visible particles, was developed still later: therefore, the discovery of properties probably belonging to this category came only recently.*

I do not intend to trace in these lectures the history of these different notions. Nevertheless, for reasons of convenience, I will present them in the indicated order.

Simple observation long ago suggested to cytologists that all sorts of cells contain in their cytoplasm a variety of particles endowed with genetic continuity. To this class of intracellular elements were considered to belong centrioles, which, in most animal and some plant cells, can be seen at the poles of the mitotic spindles; blepharoplasts and kinetosomes, which can be found at the base of cilia and flagella of many micro-organisms and of ciliated and flagellate cells of Metazoa; the kinetoplasts of Trypanosomes; mitochondria and chondriosomes, ubiquitous elements apparently present in any living cell, whether of animal or plant origin, plastids of several sort (chloroplasts, leucoplasts), assuming various functions in plant cells and in some Flagellates. Genetic continuity was ascribed to all these cytoplasmic elements because they have never been seen to arise *de novo*, that is, otherwise than from pre-existing elements of the same sort. Indeed, some cytological pictures suggest that elements of this kind multiply, like the cells themselves, by direct division Just to give you some examples, I have selected a few figures. Fig. 1 represents the amazingly complex and constant system of kinetosomes underlying the ciliary system of the protozoon *Gymnodinioides inkystans*

* There is no possibility of distinguishing, on the basis of crosses, between cytoplasmic inheritance and some other form of extrachromosomal heredity However, the involvement of the cytoplasm appears to me most probable in view of its highly organized state.

in the masterly description of Chatton and Lwoff;[2] and Fig. 2 shows how, in one of the stages of the complicated life-cycle of this animal, one row of kinetosomes, through division, gives rise to another row of similar particles. I can only mention here that the kinetosomes are polyvalent elements which give rise to a variety of structures (in *Trichomonas*, for example, the same kinetosome can give rise to a flagellum, a fibre, a parabasal body

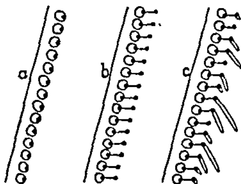


FIG. 2. Division of kinetosomes of *Gymnodinioides inkystans*. The normal row of kinetosomes (*a*) divides, giving rise to trichocystosomes (*b*), which later produce trychocysts (*c*) (from Chatton and Lwoff, 1935, see Ref. 2).

and an axostyle),* and which play an important part in the morphogenesis of Protista.[14, 25, 40] Although the claim of genetic continuity of the kinetosomes is based solely on the interpretation of the cytological pictures, one must admit that the evidence, as far as it goes, is extremely impressive.

Fig. 3, due to another very fine observer, Fauré-Fremiet, shows on the left mitochondria of the ciliated protozoan *Spirostomum ambiguum* in what is believed to be the process of division.[13] The case of mitochondria, as will appear to you later, is particularly interesting in several connexions. At this point I will only mention that genetic continuity of these elements, as well as their role as bearers

* The kind of structure to which a kinetosome gives rise depends, according to Lwoff,[25] on the quality of the particular region of cortical cytoplasm in which it is embedded.

of hereditary properties, was postulated many years ago by Meves, on the basis of cytological observations. Unfortunately, attempts at proving the point experimentally have thus far not been conclusive.

Experiments have on the contrary provided convincing proof of the genetic continuity of the kinetoplasts of Trypanosomes (Fig. 4). These disk-shaped bodies, which

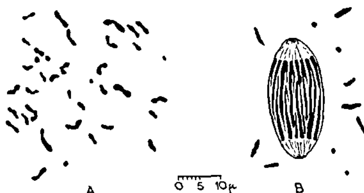


FIG 3 A Division of mitochondria of *Spirostomum ambiguum*. B Simultaneous division of mitochondria and micronucleus of *Urostyla grandis* (redrawn from Fauré-Fremiet, 1910, see Ref 13).

can be observed at the bases of the flagella, just below the kinetosomes, are curious structures which, like the nuclei, take the Feulgen stain,[24] characteristic of desoxyribonucleic acids, and are reproduced by simple division, which occurs prior to the cytoplasmic fission and about simultaneously with the division of the kinetosome (Fig. 4 A and B). It was observed many years ago by Werbitzki[41] that when Trypanosomes are grown in the presence of certain acridine dyes, the division of the kinetoplasts is blocked, while that of all the other structures proceeds undisturbed (Fig. 4 C). As a result, fission of organisms in the presence of these dyes gives rise to unequal pairs of animals, one of which is devoid of the kinetoplast (Fig. 4 D). This loss of the kinetoplast is irreversible. It is thus evident that these cell elements are endowed with genetic

continuity. Aside from this experimentally induced loss of the kinetoplast, spontaneous loss no doubt occurs from time to time: such must be the origin of akinetoplastic races of Trypanosomes which occur as parasites in certain animals.[15, 16]

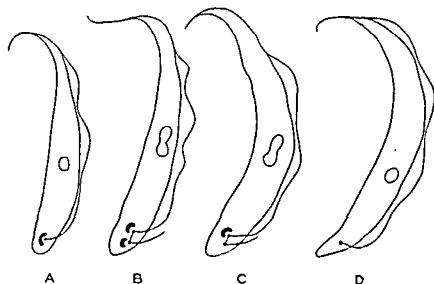


FIG. 4. Effect of acriflavine on a dividing trypanosome. The division of the kinetoplast of a normal Trypanosome (A) is shown in B. C shows the absence of division of the kinetoplast in the presence of acriflavine. D, the resulting akinetoplastic individual (redrawn from Lwoff, 1949, see Ref. 24).

The physiological function of the kinetoplast is not known. Since animals can survive its loss, it is evident that either it plays no essential part in metabolism, or that organisms devoid of these elements have some alternative metabolic pathways, which permit them, so to say, to by-pass the function of the kinetoplast.

That the latter interpretation is the correct one is made rather probable by the observations of Robertson on the flagellate *Bodo caudatus*.[32] This organism also possesses a kinetoplast, shown in Fig. 5 A, which either degenerates, or fails to divide in the presence of acriflavine (Fig. 5 D), giving rise to akinetoplastic individuals like the one shown in Fig. 5 D. Robertson did not succeed, however, in

establishing a permanently akinetoplastic clone. It thus appears probable that the kinetoplast is involved in an essential function and that, in the species studied by Robertson, there is no alternative metabolic pathway which permits compensation for the loss of the kinetoplast.

A very similar situation exists in the case of the chloroplasts of certain Flagellates. These cell elements also

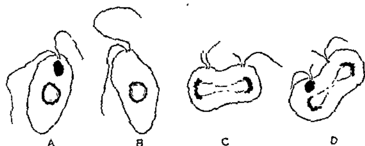


FIG 5 The origin of akinetoplastic *Bodo caudatus* A, normal type; B and C, akinetoplastic individual and its division; D, division of a normal *Bodo* in acriflavine producing one normal and one akinetoplastic individual (redrawn from Robertson, 1929, see Ref. 32).

multiply by direct division, but they do enjoy a certain autonomy with respect to the rest of the cell: the rhythm of their multiplication does not necessarily follow that of the organism as a whole. Thus, for example, when *Euglena mesnili* is cultivated in the dark, the division of the chloroplasts is slowed down and this process results in the decrease of the average number of chloroplasts per organism, as shown in Fig. 6. Eventually, organisms arise which are devoid of chloroplasts. This loss of the chloroplasts is irreversible. However, no achloroplastic clone could be established because the organisms with no chloroplasts are apparently incapable of prolonged multiplication. The loss of chloroplasts can also be induced in this species by growing it in the presence of streptomycin, but achloroplastic organisms obtained in this way cannot multiply either. It is thus clear that the loss of the chloroplast is accompanied in this case by the loss of an essential

function, and it can be shown that it is not the lack of photosynthesis which is the cause of the death of the organisms devoid of chloroplasts.[26]

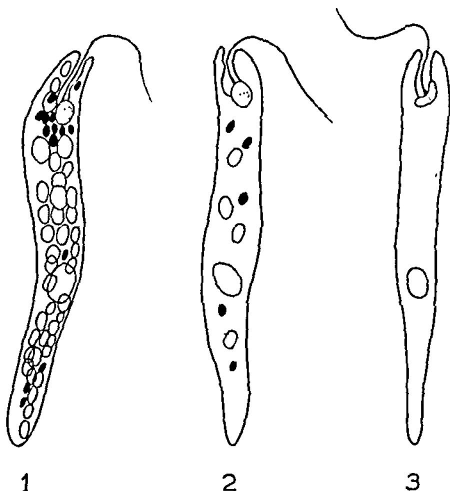


Fig. 6. *Decrease of the number of chloroplasts in Euglena mesnili grown in the dark.* 1, *Euglena* grown in light; 2, same after a short time of growth in the dark; 3, achloroplastic individual (redrawn from Lwoff, 1949, see Ref. 24).

Treatment of *Euglena gracilis* with streptomycin, although it also results in the development of permanently 'bleached' organisms, gives somewhat different results.[31] The bleaching is the result of blocked chlorophyll synthesis, that is, of the alteration of a function of the chloroplast.

But the chloroplast does not seem to disappear altogether: its other functions probably remain unaltered and this permits the continuous reproduction of bleached organisms.[25]

In the light of these results the chloroplasts appear to be complex structures, possibly comprising independent components in charge of several different functions and endowed with genetic continuity.[26]

The case of chloroplasts, apart from providing an example of autonomous cytoplasmic elements endowed with genetic continuity, is particularly instructive because it shows to what extent the demonstration of such cell elements depends on the ability of organisms to survive the loss or alteration of the elements in question. The importance of this point will appear to you later, when we discuss the intriguing problem of the rarity of proofs of cytoplasmic heredity, which is in such striking contrast with the considerable number of self-reproducing cytoplasmic elements.

3. CYTOPLASMIC HEREDITY IN YEASTS

From Flagellates we now turn our attention to yeasts, organisms which, I am sure, all of you know and appreciate. Yeasts have served Science well in the past, thanks mainly to the discoveries of the great Pasteur. You will be amused to hear that Pasteur undertook the study of yeasts for patriotic reasons. 'I was inspired in these investigations by our misfortunes', says Pasteur in the Introduction to his classic book *Études sur la Bière*. 'I undertook them directly after the 1870 war and continued them relentlessly ever since, with the resolution of carrying them far enough to stamp with a lasting progress an industry in which Germany is superior to us.' I am afraid that in spite of Pasteur's efforts, German beer remained much better than French beer. Meanwhile,

however, Pasteur's studies on yeast have laid the foundation of modern biochemistry.

Ever since Pasteur's day yeast has been one of the most frequent objects of biochemical studies and I doubt that any other organism is as well known today from the biochemical point of view. Genetical investigation of yeasts, on the contrary, began only very recently, mainly because their life-cycle was not understood. It is known today, thanks to the works of Kruis and Satava[17] and of Winge,[42, 44] and I will begin by giving you a brief account of the life-cycle of baker's yeast (Fig. 7).

A culture of baker's yeast (*Saccharomyces cerevisiae*) is composed of oval cells which are diploid and multiply by budding, a process in which a small protoplasmic bulb grows out of one side of the cell. The nucleus divides and one of the daughter nuclei migrates into the bud, which is then walled off from the mother cell before it reaches the adult size. This vegetative reproduction can go on for very prolonged periods of time and sexual reproduction does not intervene usually until the conditions for vegetative multiplication become unfavourable. The cell wall then apparently thickens and, within the at first undivided cytoplasm, the nucleus undergoes two meiotic divisions, resulting in the formation of four haploid nuclei. The cytoplasm then condenses around each of the four nuclei to form four spherical masses: we have now what is called an ascus with its four haploid ascospores which can function as gametes.

The equivalent of fertilization sets in when the asci are placed in favourable conditions. It most frequently takes one of the following two courses (Fig. 7). The ascospores swell by absorption of water and either fuse to form what is called a *spore zygote* (Fig. 7. 1) or germinate to produce a few round haploid cells which fuse in pairs, forming a *cell zygote*. Cell zygotes can be formed by fusion of two cells produced by the same or by two different ascospores (Fig. 7. 2 and Fig. 7. 3). In any case, the result is a diploid

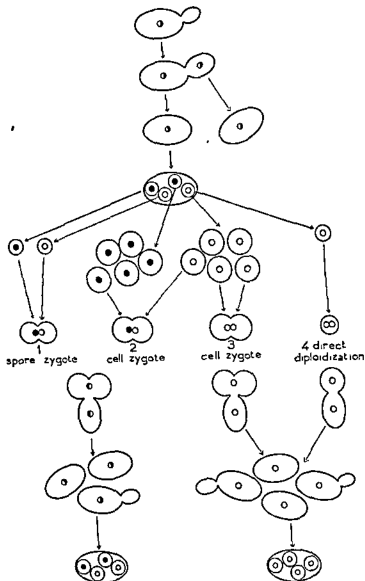


FIG 7. The life-cycle of *Saccharomyces* The figure is to be read downwards In the upper part of the figure a diploid (oval) cell is shown in the process of budding. Immediately below is shown an ascus containing four haploid (round) ascospores. Further down can be seen the four ways of diploidization which lead back to the diplophase.

zygote, which soon produces oval diploid cells by budding. In very rare cases this result is achieved in a more direct way called *direct diploidization* (Fig. 7. 4). The spore nucleus divides and the two daughter nuclei immediately fuse to form a diploid nucleus: the spore then germinates, giving rise to a diploid cell.[43, 45]

All these methods of diploidization occur only in yeast species which we call *homothallic*, that is in species in which all spores are of the same physiological type, so that any spore can fuse with any other. In other yeasts, however, as shown by experiments of Lindegren,[20] each ascus contains two spores of one mating type (+) and two of the other (−).

In these so-called *heterothallic* yeasts, copulations normally take place only between spores or cells of opposite mating types. Imagine that in Fig. 7 the black and white nuclei represent nuclei or genes of opposite mating type. It is then seen that, of the three frequent ways of diploidization shown in Fig. 7, only 1 and 2 are open to heterothallic yeasts, unless, of course, mutation of the mating-type genes takes place.

You will notice that in the species I am talking about the haploid phase is confined to a very small part of the cycle. However, the artificial isolation of single ascospores here permits the establishment of relatively stable haploid cell lines. Using two such strains of opposite mating type, one can at a chosen moment obtain fusion by mixing the two cultures. One thus obtains a new diplophase, the cells of which are capable of sporulation.[21]

This brief description of the life-cycle of baker's yeast must be supplemented by a few comments on the transmission of hereditary traits. When two haploid strains differing by a pair of alternative morphological or biochemical characters are crossed, the transmission, in the enormous majority of cases, is Mendelian: it follows the same pattern as the transmission of mating type already

illustrated in Fig. 7. The black and white circles can be taken to represent either the alternative mating type genes or any other pair of genes. The nuclei of the two haploid strains having fused in the zygote, the diploid cells formed by the zygote are heterozygous and exhibit the dominant trait. At meiosis the genes responsible for these traits segregate: if the alternative characters of the two crossed strains are due to a single gene difference, each ascus contains two spores of one type and two spores of the alternative type. When the distinguishing characters are controlled by more than one gene pair, the segregations are somewhat more complex.[18, 19, 46] I will not give you now more precise information, since I will have to return to this point later; all I want to say at this moment is that, in any case, we know exactly what segregation is expected to occur if a character is controlled by two, three, or more genes, whatever the interactions between these genes. As a corollary we also know what behaviour of pairs of characters is incompatible with the Mendelian mode of transmission, that is, with inheritance through nuclear genes.

I will now turn to cases in which such abnormal, non-Mendelian behaviour was observed.

The first of these cases was described by Winge and Laustsen.[45] It concerns the germinative power of the spores of the homothallic variety *ellipsoideus* of *S. cerevisiae*. In the original strain 68 per cent. of the spores germinate and produce viable clones. However, when the new diploid strains formed from single ascospores are studied, it is found that the germinative power of the next spore-generation depends on the mode of origin of the particular diploid line. This dependence is shown in the comparison between diploids formed within a clone derived from a single ascospore by cell zygote formation on the one hand, and by direct diploidization on the other (Fig. 8). Although in both cases fusion is between identical nuclei and therefore leads to the formation of identical homozygous diploids, the first of these produces roughly 40 per cent. of viable

spores, the second 0 to 6 per cent. Fig. 9 shows that the loss of the germinative power of these spores is permanent and cannot be restored by the intervention of another method of diploidization. Taken together, these facts indubitably point to the operation of a non-chromosomal

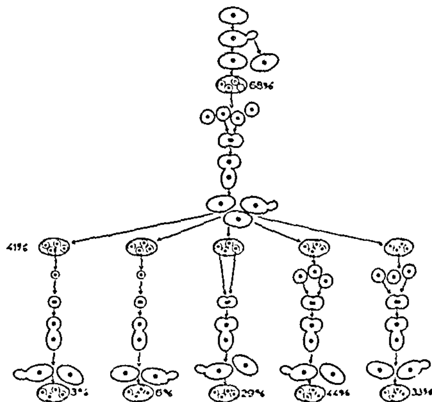


FIG. 8. Effect of different modes of diploidization (schematized as in Fig. 7) on the germinative power of ascospores (after Winge and Laustsen, 1940, see Ref. 45).

hereditary mechanism. Winge and Laustsen therefore *suggested that the difference between these diploids may* reside not in their nuclei, but in their chondriosome content. Assume that the chondriosomes divide once per cell cycle, shortly after the nuclear division. Since, in direct diploidization, the two nuclei resulting from mitosis of the spore nucleus immediately fuse to form a diploid nucleus, the division of the chondriosomes may be

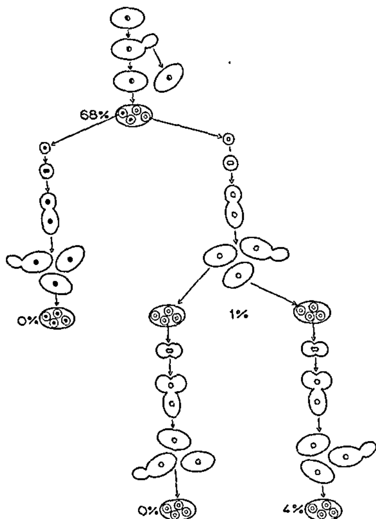


FIG. 9 Loss of germinative power of ascospores following 'direct diploidization' (after Winge and Laustsen, 1940, see Ref. 45).

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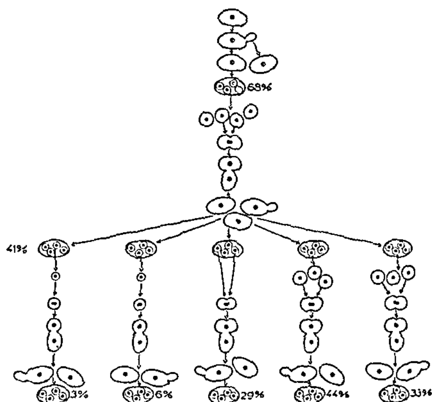


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suspended: their number will be unchanged in the diploids produced by direct diploidization. In cell zygotes, on the contrary, it is doubled, since in this case each of the haploid cells contributes its chondriosome content (Fig. 10). Winge and Laustsen thus are led to ascribe to the chondriosomes of the yeast cell the quality of autonomous cytoplasmic elements, the multiplication of which is closely correlated with nuclear division.

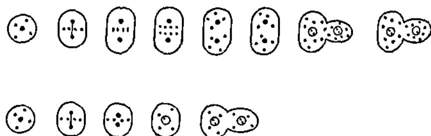


FIG. 10. Hypothetical explanation of the origin of different chondriosome contents in diploid yeasts produced by 'cell-zygote' formation (upper row) and by 'direct diploidization' (lower row) (after Winge and Laustsen, 1940, see Ref. 45).

This interpretation is, of course, hypothetical, but it is not entirely unfounded: you will remember what has been said earlier concerning the genetic continuity of mitochondria (cf. Fig. 3 B). Moreover, as you will presently see, this idea recently gained some support in the discovery of another case of cytoplasmic heredity in yeast, the study of which has kept several of my co-workers and myself busy for the last six years. Fortunately, it does not take that long to tell the story, and I hope I can give you its essence in the remainder of this lecture hour.

When a culture of baker's yeast, whether diploid or haploid, is plated, that is, spread on the surface of a solid nutrient medium in a Petri dish, each of the cells gives rise in the course of the next few days to a colony. The great majority of these colonies are of very nearly identical size, but one usually finds also a very small number—say 1

or 2 per cent.—of distinctly smaller colonies: their diameter is only one-third or half of the diameter of the bigger colonies (Fig. 11 A). These facts suggest that the population of cells which was plated was heterogeneous and that it may be possible to purify it by taking cells from either the big or the small colonies only. The results of such a selection show, however, that cells from the big colonies, when suspended in liquid and replated on nutrient agar, again and again produce the two types of colonies, while the cells from small colonies give rise to small colonies only. No selection within the big colonies can change this result: obviously these colonies are composed of cells which, in the course of their vegetative reproduction, constantly give rise both to cells similar to themselves and to cells of a different type, characterized by the small size of the colonies they form. These latter cells, on the contrary, apparently never give rise to cells different from themselves: they represent a stable type which may be regarded as the result of a hereditary variation, that is, of a mutation. Although the individual cells of the two types can hardly be distinguished by their size, I will speak of 'big' and 'little' cells, and, in order to emphasize the origin of the latter in the course of vegetative reproduction, I will call them 'vegetative littles' or, alternatively, 'vegetative mutants'.[9]

The statement that the 'littles' represent a stable cell type is based on the fact that many strains of littles have been kept by serial passages over thousands of cell generations without reverting to the original (normal) type. It must be pointed out, however, that when cells of a number of small colonies, formed on plates of a *normal* yeast, are isolated and sub-cultured, it is found that some of the clones are rather quickly invaded by normal cells which, under the usual culture conditions, enjoy a considerable selective advantage. Although this points to the possible existence of 'reversible littles', the origin of the normal cells in these clones remains uncertain. Most yeast colonies are initiated by groups of several cells rather than by single cells, and it is possible that some of the

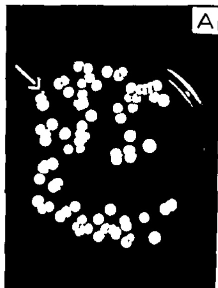




FIG. 11. Colonies formed by baker's yeast on a solid medium A, colonies of a normal yeast, showing one small colony (arrow), B, colonies formed by the same yeast grown prior to plating in the presence of acriflavine.

established by the examination of their spectra (Fig. 12). Thus the experimenter, in order to establish the normal or mutant character of a yeast strain, has at his disposal several means: the observation of the colony size, the observation of the spectrum of the yeast, and the determination of the respiratory quotient. Still a fourth means

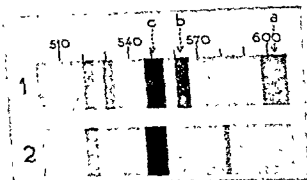


FIG 12 Spectra of normal (above) and respiration-deficient mutant yeast (below)

is the so-called Nadi reaction—a colour reaction characteristic of indophenoloxidase, which is probably identical with cytochrome oxidase. Indophenoloxidase is present in normal yeast and absent in the mutant yeast: the Nadi reaction performed on a suspension of normal cells gives a deep blue colour, and gives no colour when performed on the mutant yeast.

Slonimski[31] has performed a very thorough comparative study of the physiological properties of normal yeast and of vegetative littles. In brief, the most prominent characteristics of the mutant cells are (*a*) the total absence of cyanide-sensitive respiration, and the presence of cyanide-resistant respiration, and of aerobic fermentation; (*b*) the almost complete abolition of endogenous metabolism, (*c*) a fermentation which is equally active in the presence and in the absence of oxygen, and which, compared with the aerobic fermentation of normal yeast-cells, is much more sensitive to monoiodoacetic acid and much less to fluoride.

'reversions' are due to the inclusion, in the midst of a small colony, of a few temporarily inhibited normal cells.[9, 12]

Before we go on to the study of the genetic nature of the littles, a few words should be said about the reasons which cause the mutant type of yeast to grow more slowly than the normal yeast. It must be said first of all that this difference is observed only when the two cell types grow in the presence of oxygen; in its absence, on the contrary, their rates of growth are similar. As you certainly know, yeast can derive the energy required for its growth from the utilization of sugar in two ways: by respiration, when oxygen is present, or by fermentation when oxygen is absent. You certainly know also that the utilization of sugar is much more efficient when the respiratory path is used: for a given amount of glucose, five times more living matter is formed in aerobiosis than in anaerobiosis. The fact that the mutant yeast grows more slowly than normal yeast in aerobiosis suggests that something is wrong with its respiratory mechanism and that its fermentative mechanisms are normal.[33] This can be confirmed directly by measuring the respiratory quotient of the two cell types: this quotient is found to be of the order of 1 in normal yeast and near infinity in the mutant yeast: in other words, the respiration of the mutant yeast is nearly abolished.[33]

The causes of the respiratory deficiency of the mutant yeast have been thoroughly investigated[34, 35] and it has been established that it is due to the loss of the ability to synthesize a whole series of respiratory enzymes. Among these, the loss of two enzymes of the cytochrome system —succinic dehydrogenase and cytochrome oxidase—is particularly interesting because the presence of these enzymes in normal cells is accompanied by the occurrence in the spectrum of these cells of two characteristic bands (cytochromes *a* and *b*). These bands are absent in the mutant yeast. The difference between two cultures of yeast, one normal, one mutant, can thus be directly

two out of a thousand buds formed by normal or big cells give rise to 'vegetative mutants'. This is a mutation-rate much higher than that of the usual gene mutations. Although the process of mutation is thus frequent and unidirectional, yeast populations usually do not transform into populations of mutants because the high mutation rate is compensated by the selective advantage of the normal cells, that is, their more rapid multiplication. A total conversion of a population of normal yeast into a population of mutants is however possible if, for example, the yeast is grown in the presence of certain acridines.[12] These dyes appear to be very highly active and specific mutagenic agents: they enormously increase the frequency of the mutations without appreciably affecting the selection process. Thus, a culture of baker's yeast, grown for 48 hours in the presence of acriflavine, consists almost exclusively of mutant cells which on plating will give rise to dwarf colonies (Fig. 11 B). The examination of their spectra and of their respiration clearly shows that all these colonies are composed of the same type of mutant cells, which we have called 'vegetative littles'.

You will, of course, have noticed that since the percentage of mutant cells in a population is the *result* of two forces acting in opposite directions, viz. mutation and selection, a similar result, that is, the replacement of normal cells by mutant cells, would also be achieved if the acridine deprived the normal cells of their selective advantage without affecting at all the mutation rate. In fact, many modifications of populations of micro-organisms have found their explanation in such terms of modified selection. The following experiments[7] show, however, that the action of acridines *on* yeast is of a different sort. Single cells from a normal yeast strain are isolated with the help of a micromanipulator in droplets of culture medium containing a low concentration of euflavine. The budding of the cells is directly watched under the microscope and the successive daughter cells are removed from the mother

These metabolic characteristics of the mutants are the results of profound enzymatic changes in the cells as a consequence of the mutation. Apart from lacking the enzymes mentioned (cytochromes *a* and *b*, cytochrome oxidase, and succinic dehydrogenase), the mutants are apparently totally deficient in several other enzymes (cytochrome *c*, reduced coenzyme I-cytochrome *c* reductase, α-glycerophosphate-dehydrogenase) and contain reduced amounts of malic dehydrogenase linked to coenzyme I. The contents of alcohol-dehydrogenase and of cytochrome *c* are, on the contrary, higher than in normal cells. There seems to be no difference between normal and mutant yeasts in their content of catalase and of lactic dehydrogenase. Lastly, the mutant cells seem to contain two compounds not found in normal cells: cytochrome a_1 and malic cytochrome *c* reductase, independent of coenzyme I.

The modification of the enzymatic constitution of the cell is, according to Slonimski, an effect of the mutation on the protein moiety of the haemin-containing enzymes, rather than on their prosthetic groups.

Study of fractions of yeast homogenates obtained by differential centrifugation showed that the mutation causes the disappearance only of enzymes linked to particulate material of the cell. The observed differences in the lyoenzymes are of an entirely different order of magnitude and are regarded as a consequence of the cytochrome-oxidase deficiency.

Apart from providing us with practical means of distinguishing mutant cells from normal cells, this investigation of the biochemical differences between the two cell types gives a rather striking picture: the simultaneous loss of a series of different enzymes is not what would be expected to be the result of a gene mutation, for gene mutations usually result in single enzyme deficiencies.

A similar impression, that we are not dealing with genic mutants in the case of vegetative littles, is gained from the consideration of the frequency of the formation of cells of this type. The spontaneous mutation rate, that is, the frequency of the spontaneous occurrence of vegetative littles in the course of vegetative reproduction of baker's yeast, is of the order of 2 per 1,000; that is, approximately

Similar isolations are performed in the absence of euflavine. They constitute the controls.

After the isolated cells have multiplied for 24 hours, they are transferred to bigger volumes of culture medium. After 2 or 3 days of growth, the characteristics of each of the clones can be established by subjecting them to the Nadi reaction. Fig. 13 gives the results of one such experiment. It can be seen that, whereas none of the five control mother cells gave rise to mutant buds, more than one-half of the buds produced by the five mother cells multiplying in the acridine were mutants. Serial transfers of these clones and repeated tests showed that the mutant character was irreversible. Since the design of the experiment is such as to offer no possibility for selection (apart from cell mortality which has been found to be practically *nil*), it is clear that the acridine has a formidable mutagenic effect.

Using a different technique it has been shown that in the presence of purified euflavine, mutation rates close, if not equal, to 1 can be obtained:[27] practically every bud formed in the presence of the dye is a mutant bud.*

It must be added that the acridines produce their mutagenic effect *only* on actively multiplying cells; in the absence of cell multiplication there is *no* mutation.[9]

In these experiments on the isolation of single cells, the diagnostic test employed (Nadi reaction) is applied to the clone as a whole. Since a mixture of normal and mutant cells (up to 80 per cent. of the latter) gives a positive Nadi reaction, the clones classified as 'normal' may have contained a considerable proportion of

* The mutation rate is defined as the ratio $N_p/(N_s+N_p)$, where N_s and N_p are, respectively, the numbers of normal and mutant buds formed by a certain number of normal cells, that is, as the probability of a bud taken at random to be mutant. The justification of this definition has been given by Marcovich.[27]

Mutation rates, thus defined, approach the value of 1 in concentrations of purified euflavine which have practically no toxic effect, as measured by the lengthening of their generation time.[27]

cell, in the order of their formation, and transferred to droplets of normal medium. Similarly, second-generation

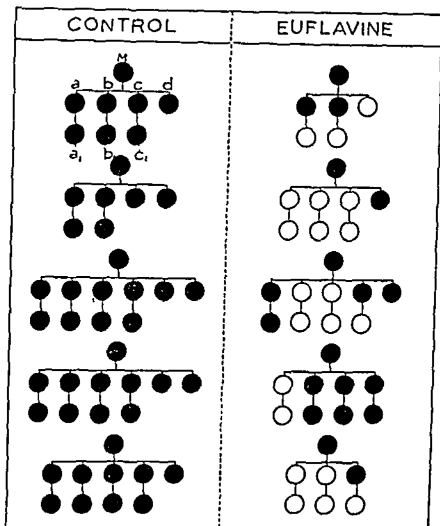


FIG. 13. Descendants of isolated yeast cells proliferating in the presence or absence of euflavine. M, mother cell; a, b, c, &c., successive buds formed by the mother cell; a_1, b_1, c_1, &c., successive second-generation buds. Black and white circles indicate normal and mutant cells, respectively

buds are separated from each of the daughter cells. Finally, at the end of the experiment, the mother cell itself is transferred to normal medium.

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mutants. That this is often the case can be shown by plating out the very young clones formed by the isolated buds. Many clones then give rise to various proportions of normal and small colonies. The variability of this proportion suggests that the mutants appear at various times in the history of the clones or, in other words, that the high mutation rate induced by the acridine is maintained after the transfer of the cells into normal medium. Study of pedigrees by the last-mentioned technique (plating) indubitably shows that the

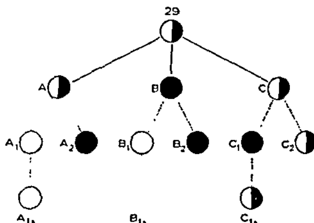


FIG. 14 Pedigree showing the instability induced in a yeast cell by treatment with euflavine. *Black, white, and half-black circles indicate cells* which have given rise to normal, mutant, and mixed clones respectively.

acridines can induce in the cells an *unstable state* which manifests itself by a long-lasting high mutation rate: the acridine-treated cell and some of its offspring, placed in normal medium, go on producing normal and mutant buds alternately over several generations. Fig. 14 gives an example of such an induced instability. Consider in particular cell C. This cell, initiated in acridine, has produced first a normal cell (C_1), then cell C_2 which was initiated in the normal medium, but which gave rise to a mixed clone of normal and mutant cells; thereafter cell C produced a clone which, when plated, developed into a mixture of big, small, and scalloped (see below, p. 34) colonies. In this case a high mutation rate was indubitably preserved over numerous cell generations.[8]

The picture of the mutation process, as it appears in the experiments I am referring to, is very different from that of induced gene mutations. As you certainly know,

gene mutations have never been observed to occur with such high rates; also, no mutagenic substance has ever been discovered, the action of which results in the appearance of invariably the same mutation. It therefore appears again very improbable that the mutants of yeast, which we are concerned with, are the results of genic mutations.

However, the two facts which I have quoted so far as suggesting that the respiratory mutants of yeast might not be due to gene mutations are, at best, presumptions. Only the usual technique of genetics, that is crossing, can permit more definite conclusions.

Let us see, then, how the mutant character behaves in sexual reproduction. Here, unfortunately, the possibilities of experimentation are limited to one type of cross. Sporulation of yeast is an aerobic process. Diploid respiratory mutants are unable to respire and therefore do not sporulate. Consequently it is impossible to establish whether the mutant character is maintained through meiosis. The only possible cross is between a normal yeast and a vegetative mutant of opposite mating type. Each of these strains carries, of course, some Mendelian 'markers': their presence permits one to ascertain that the nuclear behaviour of the hybrid is normal, for the markers show a 2:2 segregation in each ascus.

The yeast used in this work[10] was 'Yeast Foam', an American strain of diploid *Saccharomyces cerevisiae*. From an ascus of this yeast *two* spores were isolated which gave rise to two haploid strains of opposite mating type One (276/3 *b*) is adenine independent (*A*), thiamine *dependent* (*t*) *and of mating* type —. The other (276/3 *d*) is both adenine and thiamine independent (*AT*) and of mating type +. Both strains are normal with respect to respiratory mechanism ('big')

Among the colonies formed by the first of these strains on an agar plate, a red colony was found. It is the origin of a strain of red yeast (276/3 *br*) which, like the yeast clone within which it arose, is haploid, thiamine dependent and of mating type —. However, contrary to the latter, it is adenine dependent (*a*). Both

mutants. That this is often the case can be shown by plating out the very young clones formed by the isolated buds. Many clones then give rise to various proportions of normal and small colonies. The variability of this proportion suggests that the mutants appear at various times in the history of the clones or, in other words, that the high *mutation rate induced by the acridine is maintained after* the transfer of the cells into normal medium. Study of pedigrees by the last-mentioned technique (plating) indubitably shows that the

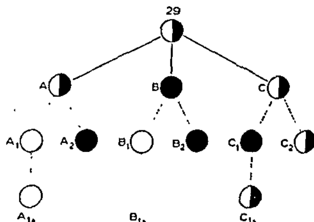


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mutation of a single gene. No conclusion beyond this can, however, be drawn, since it must be kept in mind that if the respiratory deficiency were due to the simultaneous mutation of several genes to their recessive allelomorphs, the mutant character would reappear only in a fraction of

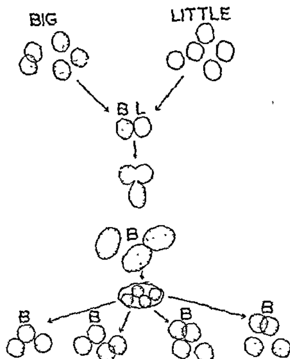


FIG 15 Results of a cross between a normal strain and a 'vegetative mutant' of yeast The cytoplasm of the normal cells is indicated by stippling.

asci, the proportion of which would depend on the number of genes involved: the greater the number of recessive genes involved, the smaller the proportion of asci containing mutant spores should be. Just to give you an example: if the respiratory deficiency were due to the simultaneous presence of four recessive genes, it would reappear only in some 12 per cent. of the asci. The fact that the mutant character was not observed to reappear in

the adenine dependence and the red pigment are effects of the same recessive *a*.

The red yeast, like the strain of origin, possesses a normal respiration. Like any other strain of yeast, it frequently gives rise to vegetative littles, the frequency of which is increased by growth in the presence of acridines: strain 1*A*-2 involved in the cross to be described was obtained from such treatment. An interesting and technically convenient feature of the vegetative mutants of the red yeast is that, although they contain the recessive gene *a*, they form white colonies when grown in the usual manner on media containing 2–3 per cent. glucose.

All crosses to be described now were made between pairs of haploid yeast cultures of which one is *AT*+ and the other *at*−. Every spore isolated from the asci of the hybrid was tested for these markers. All asci showed a 2:2 segregation of the mating type genes. A 2:2 segregation for genes *A*/*a* was observed in 129 out of 134 asci. A similar segregation for the pair *T*/*t* was observed only in 120 out of 134 asci. This rather poor result is ascribed to imperfection of the technique of testing. The important point is, however, that all the asci contained both *A* and *a* and *T* and *t* spores; in other words, they were 'legitimate' hybrid asci.

The results of the cross between normal yeast and a 'vegetative little' are summarized in Fig. 15. The fusion of the two cell types results in the formation of diploid cells which are normal with respect to respiration. These diploid cells are able to sporulate and the genetic analysis of the asci is therefore possible, by the isolation of single ascospores. Such analysis shows that all four spores of the hybrid asci give rise to clones with normal respiration. In other words, while the genetic markers undergo perfectly normal segregation, the mutant character we are interested in, that is the respiratory deficiency, vanishes on crossing and does not reappear in the spore progeny.[10]

If the respiratory deficiency were the result of the mutation of a single gene, it should reappear, like the genetic markers, in two of the four spores of each ascus. Since this is not the case, we may conclude that the respiratory deficiency of the vegetative littles is not caused by the

incredibly high, mutation rates of individual genes have to be assumed on the polygenic hypothesis. Thus, the results of the crosses support the presumptions quoted earlier against the interpretation of the respiratory deficiency in terms of mutations of Mendelian genes and point to the intervention of an extrachromosomal factor. That is why I some years ago[5] adopted, as a working hypothesis, an interpretation which assumes that *the vegetative littles are due to a cytoplasmic, rather than a nuclear mutation.** This interpretation postulates that the synthesis of the respiratory enzymes, missing in the mutants, is dependent upon the presence of a cytoplasmic factor, which is self-reproducing and particulate. If it is further assumed that this factor is distributed at random between the mother cell and the bud when the latter is formed, and that the average number of the hypothetical particles per cell of the normal strain is rather low, say of the order of 10, it is easy to see how some buds happen to contain no particles at the moment they are walled off from the mother cell: such buds, containing no particles and consequently unable to synthesize the respiratory enzymes, are, in other words, the result of a loss mutation. The action of the acridines can be accounted for by supposing that these substances electively affect the cytoplasmic particles in a manner analogous to that in which they act on the kinetoplasts of Trypanosomes, that is, either by destroying them or by preventing their multiplication. (One could, of course, assume that the cytoplasmic particles are not lost in the mutants, but that they have mutated to an inactive condition. No test permits one at present to distinguish between loss and inactivation.)

If a suspension of normal yeast is plated on a solid medium containing at least one part in 300,000 of acriflavine, all the cells give rise to small colonies, composed of respiration-deficient mutants.

* Lindegren and Lindegren[33] have observed very similar results with another strain of red yeast. Their interpretation is, however, different from the one proposed below.

the spore progeny of the hybrid may therefore be due simply to the necessarily small number of asci which it is possible to analyse in practice, and the possibility must be recognized that different results might have been obtained *if a much greater number of asci had been studied*. This difficulty can obviously be removed by the use of forced labour, but fortunately less drastic methods can achieve the same result: one can make use of the well-known technique of backcrossing, that is, of repeatedly crossing the spores formed by the hybrid with the mutant parent. In this way the genes of the mutant parent accumulate in the hybrids of the successive generations. Consequently, if the mutant character required the simultaneous presence of several recessives to manifest itself, the proportion of asci containing mutant spores should rapidly increase in the course of the successive backcrosses.

This method has indeed been used[10] to follow up the results of the first generation which I have stated earlier. Five successive backcrosses have been made, but the result expected on the basis of the polygenic hypothesis was not achieved.* A few mutant spores did occur in the course of the backcrosses, but their frequency was not greater than the frequency of spontaneous mutations, and calculations have shown that, in order to interpret the experimental results in terms of Mendelian genes, one would have to assume that the manifestation of the mutant character under consideration depends on the simultaneous presence of at least a dozen recessive genes.[10] This does not, of course, prove that the genic interpretation is wrong: it merely makes it very unlikely. As I have pointed out earlier, the mutation rate we would have to assume to account for the frequency of the spontaneous occurrence of vegetative mutants if they were due to changes of single genes is unusually high; but still higher, and in fact

* The actual number of asci analysed in the first cross and in the five successive backcrosses are, respectively: 31, 23, 18, 20, 57, and 6, making *a total of 620 spores. Among these, five were mutant.*

the enzymes lacking in the mutant are linked to particulate material separable by differential centrifugation.[34,35] The enzyme-carrying particles, sometimes called macrosomes, can probably be identified with mitochondria. The idea therefore naturally suggests itself that the hypothetical particles, postulated on the ground of genetic experiments, are in fact mitochondria, and that the mutation which results in the formation of vegetative littles consists of the loss of mitochondria.[6,35] Microscopic observation of normal yeast cells on which has been performed the Nadi reaction, characteristic of indophenoloxidase (one of the enzymes missing in the littles), shows that the colour is concentrated in a small number of cytoplasmic granules. Each cell contains many granules, but only some of them show the blue colour. The cytoplasm of the mutant cells also contains such visible granules, but none of them are coloured. It is tempting to assume that the blue granules of the normal cells *are* the particles postulated on genetic grounds. It is particularly tempting because, if proved, it would explain, without resort to other factors, the simultaneous disappearance of the several different enzymes present in normal cells and absent in the mutants. It must be admitted though that, however tempting, such conclusions are not justified at the present stage. First of all, there is at present no proof that the indophenol blue is actually produced in, rather than adsorbed on, the visible granules. Secondly, were this proven, one would be entitled to say that the visible granules carry the indophenoloxidase, but the identity of the enzyme-carrying elements with the hypothetical gene-like particles would still require demonstration, which so far has been beyond our reach. *The only conclusion we may draw today with a high probability of being right is that the normal yeast and the vegetative mutants differ by the presence in the former and the absence in the latter of cytoplasmic units endowed with genetic continuity and required for the synthesis of certain respiratory enzymes.*

If the concentration of acriflavine is slightly lower (one part in 500,000), all the colonies are big, *but have a characteristic irregular* contour. Similar 'scalloped' colonies develop when a yeast culture, or isolated cells (like those in the pedigrees described on p. 28), are grown for a short time in liquid medium containing mutagenic acridines, and are then plated on normal nutrient agar.

When a 'scalloped' colony is dissociated and replated, it gives rise to a mixture of normal and small colonies. Since normal cells possess a distinct selective advantage, the formation of scalloped colonies is ascribed to the unstable state of the cell from which the colony originated (see above, p. 28). This cell gives rise alternately to similar unstable cells, to normal cells, and to vegetative mutants, as in the pedigrees described on p. 28.

The simplest interpretation of the induced unstable state is that *it is due to the diminished number of cytoplasmic particles*, owing to destruction of the latter by the acridine. Obviously, the smaller the number of particles per cell, the higher the probability of their loss, that is, of the mutation.[8]

This interpretation has the advantage of offering also a simple explanation of the results of the cross between a normal yeast and a vegetative little. Since the cross results in the mixture of the cytoplasms of the cells undergoing fusion, the diploid cells contain the cytoplasmic factor supplied by the normal parent. Furthermore, since, in the process of sporulation, the spores are simply cut out of the cytoplasm of the ascus-forming cell, all spores contain some particles, and the mutant character of the mutant parent, caused by the total absence of particles, does not reappear in the asci.*

Can the hypothetical particles be identified with any known visible elements of the yeast cell? In normal yeast

* In view of this behaviour in crosses of the character under consideration, the opinion has been expressed that the terms 'mutation' and 'mutant' should be avoided in speaking of the *vegetative littles*. *It has been suggested that*, instead, one might speak of 'differentiation' and 'phenocopy'. However, the use of such terms appears to me undesirable, for the mechanisms of the phenomena for the description of which they have been invented are unknown, *and it is precisely my purpose to suggest that* differentiation may be due to discontinuous and irreversible changes in the heredity of cells, that is, to mutations.

national characteristics, but you will see that the behaviour of the French strain is somewhat more fanciful. Like the American strain, it produces some vegetative mutants in the course of its vegetative reproduction, but in addition it produces in its asci many mutant spores, that is, spores which give rise to clones entirely composed of cells which present the same biochemical characteristics as the vegetative littles you are now familiar with. In spite of this similarity, I am going to designate them by a different term; I will speak of 'segregational mutants': you will soon see that this distinctive is justified.

The germinative power of the ascospores of this French strain of yeast is very poor: only 4 per cent. of all the ascospores germinate to produce viable clones. It is therefore impossible to determine in what ratios the mutant spores occur in the asci. It was found, however, that the frequency of mutant spores among all the viable spores was close to 50 per cent. This suggested that the cells of strains B-II might be heterozygous for a recessive Mendelian gene controlling the character of the spores. This was worth investigating because, if this were the case, it would demonstrate that the respiratory deficiency can be caused by the mutation of a nuclear gene as well as by the loss of cytoplasmic particles; and this in turn might indicate a relation between these two sorts of cell constituents.

These expectations were borne out by crosses between three haploid strains derived from the French yeast. The precise relationship between these strains is shown in Fig. 16. From two asci formed by the original strain B-II, two ascospores of opposite mating type were isolated. One of these was normal and gave rise to a clone of haploid cells (B 15) normal with respect to respiration; the other was a 'segregational mutant' and produced a clone (B 26) of mutant, respiration-deficient haploid cells. From the former (B 15) a 'vegetative mutant' (B 15p4) was isolated. In Fig. 16 the mutant cells are indicated by a smaller size.

Sturtevant has suggested in private discussion a different interpretation which likens our 'vegetative mutations' to the variegations observed in *Drosophila*. In Sturtevant's opinion the latter are due to phenotypic inactivity of definite genes, unstable in the somatic line (the instability being caused by a translocation into the neighbourhood of heterochromatin, for example), but necessarily restored to normal activity during meiosis.

The inability of 'vegetative mutants' to sporulate has thus far not permitted this ingenious hypothesis to be subjected to experimental test.

While a certain number of most intriguing problems thus remain momentarily unanswerable, we may turn to others and, in particular, to the question of the relation between the postulated cytoplasmic genetic units of the yeast cell and its nucleus. Are these units totally independent of the nucleus in their reproduction and their function?

Although all the experiments thus far described favour the view that they are completely autonomous, you will see in a moment that such *autonomy* can never be safely claimed, by contrast to *lack of autonomy* (that is dependence) which can be directly proved. However, you will see also that the demonstration of the dependence of any cell element on the nucleus is entirely subject to the accident of discovery of a nuclear constitution which interferes with the normal multiplication or the normal functioning of the element in question. Consequently, an apparent autonomy can always be ascribed to the fact that the proper nuclear constitution has not yet been discovered.

All this will become clearer to you after I have told you the results of some other observations. The experiments I have described so far were all performed on an American strain of yeast, called Yeast Foam; in the experiments I am going to describe now,[a] a strain of French baker's yeast, belonging to the same species and called B-II, was used. I do not want to imply anything about

Between these three strains two crosses are possible: the 'segregational mutant', of mating type −, can be crossed with each of the other two strains, which are both of + mating type.

The cross between the segregational mutant (B 26) and the normal strain (B 15) results in the formation of diploid cells with normal respiration. These cells are therefore able to sporulate. The analysis of the asci showed that each of them contained two normal and two mutant spores. This result confirms the hypothesis that the difference between 'normal' and 'segregational mutant' is genic, and the clear unifactorial 2:2 ratio found in the asci shows that these two strains are differentiated by a single gene pair. Since the diploid cells formed as a result of the cross are normal, it is clear that the normal strain B 15 carries a dominant gene, say *R*, and that the respiratory deficiency of the segregational mutant B 26 is due to the presence of the recessive allelomorph *r*.

The second cross provided us with results much more difficult to explain in Mendelian terms. Following the cross of the same 'segregational mutant' B 26 with the 'vegetative mutant' (B 15p4), diploid cells are formed which possess a normal respiration. Analysis of the asci formed by these diploids showed that these asci too each contain two normal and two mutant ascospores. The reconstitution of a normal diploid cell by the fusion of two haploid mutants can easily be explained if it is assumed that each of the strains crossed owes its mutant character to the mutation of a *different*, non allelic, gene; but the sporulation of such a doubly heterozygous hybrid should, you remember, result in segregation ratios more complex than the typically unifactorial 2:2 ratio actually observed.

If the two crossed strains of littles owed their character to the presence of two different *non-allelic* recessives, the expected segregations would be 0:4, 1:3, and 2:2 in different asci. If the two genes in question were not linked, the proportion of asci of the 1:3 type would depend on the distance of the genes from the

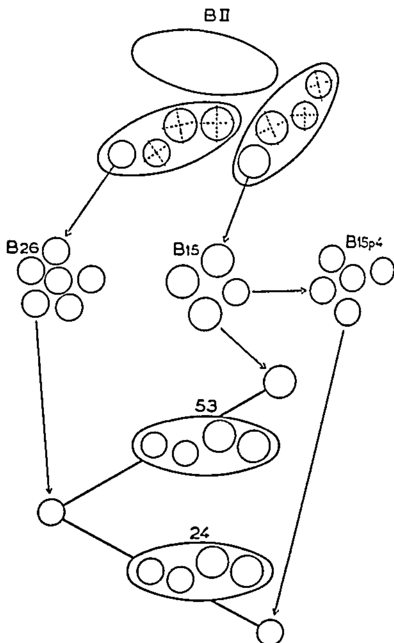


FIG. 16. Results of crosses between three strains of yeast B-II. The upper part of the figure shows the origin of the three haploid strains B 15 is a yeast with normal respiration, B 15p4 and B 26 are respectively clones of 'vegetative' and 'segregational' littles The mutant phenotype is indicated by the small size of the cells

enzymes with which we are concerned are distinct entities. On the other hand, the question whether the enzyme-carrying par-

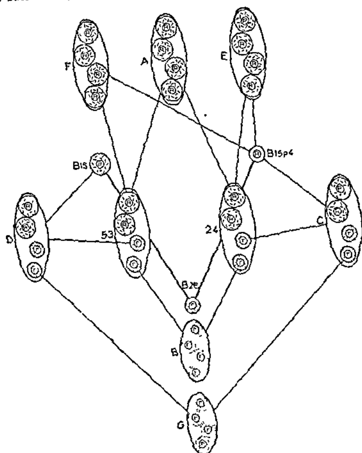


FIG 17 Results of seven crosses between strains of yeast B-II, expected on the basis of the interpretation given on p. 40. In the middle of the figure are represented the *same* two crosses as in Fig. *16* The size of the asci indicates the normal (big) or mutant (little) character of the diploid hybrids. The size of the ascospores symbolizes *their* normal or mutant character *R* and *r* are two allelic *nuclear genes. Active* cytoplasmic particles are represented as rods, inactive particles as dots.

ticles sedimentable by centrifugation and the particles postulated on genetic grounds are one and the same thing remains at present unanswered. The assumption that *they* are identical is simplest

centromeres, the rest of the asci being equally divided between the 0:4 and the 2:2 types. If the genes were linked, the frequency of 2:2 asci should be higher than that of asci of the 0:4 type.

While the experimental results are thus difficult to explain if only genic differences between the three strains are postulated, they are completely and easily accounted for by an interpretation postulating the intervention of both genic and cytoplasmic factors.

Assume that in this yeast, as in the American one (Yeast Foam), there is no genic difference between the normal haploid B 15 and the vegetative mutant B 15p4 derived from it, and, that the vegetative mutant is the result of a loss-mutation, the loss involving the cytoplasmic particles necessary for the synthesis of respiratory enzymes. Assume, on the other hand, that the segregational mutant does contain the cytoplasmic particles, but that it carries a recessive gene in the presence of which the cytoplasmic granules are physiologically inactive. Fig. 17, in which the inactive particles are represented by dots and the active ones by rods, shows in its centre the expected results of the two crosses I described (cross 53: B 26×B 15 and cross 24: B 26×B 15p4). You can see that on our assumptions both crosses should result in the formation of diploids with normal respiration because in both cases the diploids formed carry the dominant gene *R* and contain the cytoplasmic particles. They are both normal biochemically because in each of them the cytoplasmic particles are activated by the dominant gene; and they both produce asci showing a 2:2 segregation because they both are heterozygous for a single gene pair *R/r*: after meiosis the cytoplasmic particles remain active only in the two spores carrying the dominant *R*; in the two others, carrying the recessive *r*, the particles become, on the contrary, physiologically inactive.

The fact that segregational mutants manifest the respiratory deficiency although they contain the cytoplasmic particles endowed with genetic continuity proves that the genetic units and the

Crosses B and G, on the contrary, each involve two segregational mutants, that is cells containing inactive cytoplasmic particles and the recessive gene *r*. The diploids formed in these two crosses should therefore be respiration-deficient.

Examination of the data of columns 2 and 3 in Table I shows that these expectations are fulfilled.

Let us now turn to the segregations expected to occur in the asci of the seven crosses, and begin with crosses A and F which are particularly important, for they are tests of the correctness of our assumption that the normal strain (B 15) and the vegetative mutant (B 15p4) derived from it, are genically identical, both carrying the dominant gene *R*. Because these two strains are of the same mating type, we were unable to verify this assumption by crossing them. We can now bridge the gap in the demonstration: as a result of gene recombination which has taken place during spore formation in the F_1 hybrid (53) between normal (B 15) and segregational little (B 26), many of the normal spores (that is of the spores carrying gene *R* inherited from the normal parent) are now of mating type −. These spores can now be backcrossed to the vegetative mutant parent (B 15p4). Cross F is such a backcross. If the vegetative mutant contains, as postulated, gene *R*, this cross must result in asci containing four normal spores (4:0 segregation).

Cross A is important for the same reason. It is a cross between two normal clones derived from ascospores of the two F_1 crosses respectively. Each of them is supposed to have inherited the same *R* allele. Cross A is therefore expected to result in asci containing only normal spores.

A similar 4:0 ratio is expected in cross E which is a backcross of an F_1 normal from cross 24 to the vegetative mutant parent of presumably identical genotype.

Crosses C and D are backcrosses of F_1 littles from the two crosses 53 and 24 to their respective parents presumed to carry gene *R*. (In the first case this parent is

in the sense that it offers a simple mechanical explanation for the simultaneous disappearance from the mutant cells of several enzymes known to be linked to sedimentable cell components.

The suggested interpretation thus fully accounts for the observed results. The correctness of its postulates can be further checked by a number of crosses. The expected results of seven of these are given in Fig. 17 (A–G) and in the 2nd and 4th columns of Table I.

TABLE I

	Nadi reaction of diploid hybrid		*Segregation*		
*Cross**	*Expected*	*Observed*	*Expected*	*Observed*	*Nature of spores in the aberrant asci†*
53	+	+	2:2	31 (2:2)	..
24	+	+	2:2	15 (2:2)	..
A	+	+	4:0	9 (4:0)	..
B	—	—	0:4	no asci	.
C	+	+	2:2	18 (2:2)	.
D	+	+	2:2	20 (2:2)	..
				1 (0:4)	(vm vm sm dm)
E	+	+	4:0	11 (4:0)	..
				3 (3:1)	3 (vm)
F	+	+	4:0	19 (4:0)	.
				1 (3:1)	(vm)
				2 (1:3)	6 (vm)
				5 (0:4)	20 (vm)
G	—	—	0:4	no asci	..

* The designation of the crosses corresponds to that of Fig. 17.
† vm: vegetative mutant; sm: segregational mutant; dm: dual mutant.

Let us first consider what the character of the diploid hybrids of these different crosses should be according to our scheme.

Crosses A, C, D, E, and F should lead to the formation of diploids carrying the dominant gene *R* either in homozygous or heterozygous form. Since, on the other hand, all of them *involve at least one strain bringing in the* cytoplasmic particles, all diploid hybrids of these crosses should possess normal respiratory characteristics.

Crosses B and G, on the contrary, each involve two segregational mutants, that is cells containing inactive cytoplasmic particles and the recessive gene *r*. The diploids formed in these two crosses should therefore be respiration-deficient.

Examination of the data of columns 2 and 3 in Table I shows that these expectations are fulfilled.

Let us now turn to the segregations expected to occur in the asci of the seven crosses, and begin with crosses A and F which are particularly important, for they are tests of the correctness of our assumption that the normal strain (B 15) and the vegetative mutant (B 15p4) derived from it, are genically identical, both carrying the dominant gene *R*. Because these two strains are of the same mating type, we were unable to verify this assumption by crossing them. We can now bridge the gap in the demonstration: as a result of gene recombination which has taken place during spore formation in the F_1 hybrid (53) between normal (B 15) and segregational little (B 26), many of the normal spores (that is of the spores carrying gene *R* inherited from the normal parent) are now of mating type −. These spores can now be backcrossed to the vegetative mutant parent (B 15p4). Cross F is such a backcross. If the vegetative mutant contains, as postulated, gene *R*, this cross must result in asci containing four normal spores (4:0 segregation).

Cross A is important for the same reason. It is a cross between two normal clones derived from ascospores of the two F_1 crosses respectively. Each of them is supposed to have inherited the same *R* allele. Cross A is therefore expected to result in asci containing only normal spores.

A similar 4:0 ratio is expected in cross E which is a backcross of an F_1 normal from cross 24 to the vegetative mutant parent of presumably identical genotype.

Crosses C and D are backcrosses of F_1 littles from the two crosses 53 and 24 to their respective parents presumed to carry gene *R*. (In the first case this parent is

a vegetative mutant, in the second it is normal.) Both crosses are therefore expected to give 2:2 segregations.

Lastly, crosses B and G should result in asci containing four mutant spores each (0:4 segregations), since they are combinations of segregational mutants.

These are the expectations.

Comparison of columns 4 and 5 of Table I, which give the expected and observed segregations in the asci formed by the different hybrids, reveals a situation which is somewhat more complex than anticipated. Two of the hybrids (B and G) did not sporulate: this should not surprise us, however, since, as stated earlier, diploid vegetative mutants with deficient respiration are unable to sporulate. Obviously, diploid segregational mutants cannot sporulate either. More serious is the fact that in several of the other crosses, in addition to the expected segregations, some unexpected ratios were observed.

Does this mean that the hypothesis we formulated is wrong? Let me reassure you: you will see very shortly that unexpected results, as so often happens in science, are as valuable in the demonstration of the correctness of our hypothesis as expected ones: they are the exceptions which prove the rule.

You will have noticed that all the asci which I may call 'exceptional' contain an *excess* of mutant ascospores. Let us determine to what type the mutant spores of the exceptional asci belong. Following our hypothesis, this can be done in the manner shown in Fig. 18. The clone of cells derived from the ascospores to be tested (marked in the figure by a question mark) is crossed to two tester strains: one of them is a strain of vegetative mutants, the other a strain of segregational mutants. If the tested strain is a strain of vegetative littles, the first cross will result in the formation of respiration-deficient diploid cells; the second in the formation of normal diploids. If the tested strain is a strain of segregational mutants, the results of the two crosses will be reversed. Finally, there obviously is a third

possibility: the tested strain could be what I shall call a 'dual mutant', the cells carrying neither dominant gene, nor cytoplasmic particles. Such a 'dual mutant' should, according to our hypothesis, form respiration-deficient diploids in both test crosses.

Test strains: vm	Test strains: sm	Conclusion
? × R M	? × r M	
RR M	Rr N	?=vm R 1
rR N	rr M	?=sm r 2
rR M	rr M	?=dm r 3

FIG. 18 Diagram indicating the method of testing mutant ascospores found in 'aberrant' asci. N, normal phenotype; M, mutant phenotype; vm, vegetative mutant; sm, segregational mutant; dm, dual mutant. Other symbols as in Fig 17.

The results of this test, applied to all mutant spores of the exceptional asci, are given in the last column of Table I. These results clearly show that the genic segregation was in agreement with expectation in all the exceptional asci. In other words, the exceptional phenotypic ratios were in every case due to the loss of the cytoplasmic particles.

Summing up, the study of the two strains of baker's yeast shows that *the synthesis of respiratory enzymes by these organisms requires the simultaneous presence of a cytoplasmic factor and of a dominant nuclear gene.*[3] The

cytoplasmic factor appears, in the light of the experiments I have described, to be dependent on the nucleus in its function, and independent of it in its reproduction.

This statement requires, however, a qualification. While the functional dependence of the cytoplasmic particles on the nucleus appears to be established beyond doubt, their apparently complete autonomy in reproduction is deduced from purely negative evidence. We have seen how misleading this kind of evidence can be: the cytoplasmic particles appeared to be completely autonomous in their function until the investigations of strain B-II were made. Similarly, their independence with respect to the nucleus in reproduction may be only apparent.

That this may be the case is actually suggested by observations[11] on strains which present, in populations in equilibrium, very high proportions of vegetative mutants. It appears that this characteristic is due to a high spontaneous mutation rate and is controlled by a recessive gene. A cross between a normal strain and one with a high mutation rate results in a 2·2 segregation of the character. Since the mutation itself seems to consist in the loss of cytoplasmic particles, the role of the mutability-gene may be interpreted as increasing the probability of the loss.

This situation can be accounted for by assuming that the mutability-gene controls some intracellular condition which limits the average content of particles per cell to a low number. This result could be achieved if, for example, the recessive gene caused a slow multiplication of the cytoplasmic particles.

That the gene does not affect the intrinsic properties of the particles is proved by the fact that hybrids produced by the cross of a mutable strain with a vegetative little, isolated from a 'stable' strain, produces spore progeny which again segregates into 'highly mutable' and 'stable'. In other words, the cytoplasmic particles of a 'highly mutable' yeast again show normal behaviour when placed in the presence of the dominant gene.

Long-term adaptation to galactose fermentation. Winge and Roberts[46] have described in *Saccharomyces Chevalieri* strains differing in the ability to ferment galactose. This difference has been shown to be based on the presence in the fermenters of a

dominant gene *G* and of its recessive allele g, in the slowly adapting strains.

Study of the phenomenon of 'long-term adaptation' to galactose fermentation in a strain of the latter sort has led Spiegelman and co-workers[36] to the conclusion that it involves the induction, in a small proportion of 'positives' exposed to the substrate, of a cytoplasmically transmitted enzyme-forming system, distinct from the enzyme itself. This cytoplasmic factor is maintained during growth in the presence of galactose, but rapidly and suddenly disappears when the 'positives' are placed in glucose-medium. The loss is, however, not permanent, and a certain proportion of negative cells remain capable of producing the enzyme-forming system again when placed in the presence of galactose.

The kinetics of the reversion from positive to negative has been studied by bud analysis of the sort which led to the demonstration of the mutagenic action of euflavine The pedigrees obtained show a great similarity with those described above. The authors conclude that enzyme formation is dependent on a cytoplasmic particulate enzyme-forming system, randomly distributed between mother cell and bud

By subjecting galactose-adapted cells to euflavine treatment, Spiegelman and co-workers observed vegetative respiration-deficient mutants which contain galactozymase and transmit the enzyme-forming capacity to their progeny. The reversion to negatives follows in these vegetative mutants the same course as in the cells with normal respiration.

It is concluded that the particles responsible for galactozymase formation are distinct from those involved in the synthesis of respiratory enzymes.

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II

'. . . it may at first sight seem strange that so many nature lovers of the old times have been enchanted by ciliates. Of course, watching a ciliate stopping in his search for food to look at you through the microscope is most stirring. But my impression is that the lover of ciliates always had a presentiment that ciliates were to be the proving ground for visible self-reproducing particles.'

(ANDRÉ LWOFF, *Problems of Morphogenesis in Ciliates*, Wiley and Sons, New York, 1950, p. 93.)

1. THE 'KILLER' CHARACTER IN *PARAMECIUM*

THE organism I am going to talk about today is the ciliated Protozoon, *Paramecium aurelia*—known, I am sure, to all of you. This organism, almost as glorious as yeast, is of no use to human industry. It owes its fame to a series of investigations by Butschli, Engelmann, Maupas, Hertwig, Erdman, Woodruff, Calkins, and others which, at one time, seemed to promise to unveil the secrets of senescence and rejuvenescence. This, of course, soon proved to be an illusion, and the popularity of *Paramecium* was steadily running downhill when an American, as Americans sometimes do, came to its rescue. Starting in 1943, Sonneborn and his co-workers revealed the existence in *P. aurelia* of nucleo-cytoplasmic relations which placed this organism once more in the centre of the biologists' attention and—a result more difficult to achieve—awakened the interest of some geneticists in the cytoplasm.

In *Paramecium aurelia* each animal contains two sorts of genetically identical nuclei: two small diploid *micronuclei* which play a part only in sexual reproduction, and one big (probably polyploid) *macronucleus* which is the physiologically active nucleus. The animals multiply by binary fission, in the course of which all three nuclei divide. The division of the micronuclei is typically mitotic.

The sexual phenomena of *Paramecium* with which we

will be concerned are of two types, called *conjugation* and *autogamy*.

Conjugation is essentially a process of reciprocal fertilization accomplished by the exchange of nuclei between pairs of animals. Normally it is not accompanied by exchange of cytoplasm.

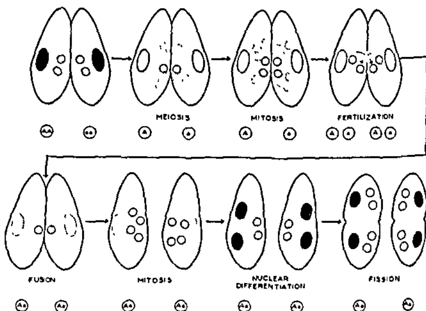


FIG. 19. Conjugation of two Paramecia of *AA* and *aa* genotypes (redrawn from Beadle, 1949, see Ref. 1).

Conjugation begins by the association in pairs of animals of opposite mating types (Fig. 19). In each of the conjugants the micronuclei undergo meiosis, thus giving rise to eight haploid nuclei. Seven of these nuclei degenerate and the remaining one divides once more by mitosis: each animal now contains two identical haploid nuclei, one of which migrates into the other conjugant. This migration is followed by the fusion of the two micronuclei into a diploid nucleus. The conjugants now fall apart. In each of them the new nucleus undergoes two mitotic divisions: two of the daughter nuclei differentiate into micronuclei, the two others into macronuclei. Meanwhile, the old

macronucleus has degenerated, and the two exconjugants thus possess two micronuclei and two macronuclei. At the first fission following conjugation, the two micronuclei divide, while the two macronuclei do not: each of the daughter cells receives one of them. Thus, the first fission gives rise to animals which, like the Paramecia we started with, contain two micronuclei and one macronucleus.

In order to show you the genetic consequences of the process of conjugation, I will now assume that the genotype of one of the conjugants is *AA* and that of other *aa* (Fig 19). As a result of the two meiotic divisions, followed by the degeneration of all but one of the eight micronuclei, each of the conjugants contains only one haploid set of genes. In one of the conjugants the nucleus contains gene *A*, in the other gene *a*. The third division of the nucleus gives rise in each of the animals to two identical nuclei: in one of them *A* and *A*, in the other *a* and *a*. After nuclear exchange, each of the animals becomes heterozygous for the two genes, that is *A*/*a*. You can see that, whatever the initial difference between the genotypes of the two conjugants, after conjugation their genotypes are identical. It is clear that conjugation is a kind of reciprocal fertilization.

When conjugation occurs between heterozygotes (*A*/*a*), each individual conjugant has equal chances (50 per cent) of containing an *A* or an *a* 'gamete nucleus' after the degeneration of seven out of the eight products of the division of the micronuclei. Fertilization therefore involves *A* and *A*, or *A* and *a*, or *a* and *a* gamete nuclei in 25, 50, and 25 per cent. of the cases respectively. Conjugation between heterozygotes thus results in the production of pairs of exconjugants which are either *A*/*A* or *A*/*a* or *a*/*a* with the relative frequencies 1 2 1 Owing to the dominance of *A*, the phenotypic ratio is 3*A* 1*a*.

In addition to this perfectly normal biparental sexual activity, Paramecia sometimes undergo autogamy, a process of uniparental nuclear reorganization: there is apparently *no moral* code among these organisms to prevent them from indulging in such solitary pleasures.

The nuclear phenomena in autogamy are very similar to those occurring in conjugation. The micronuclei of the animal undergo the same divisions and degenerations as in each of the conjugants, but no migration of the nuclei takes place. In its stead, the two identical haploid sister nuclei fuse into a diploid nucleus. As can be seen in Fig. 20, the genetic consequence of this process is that, if the

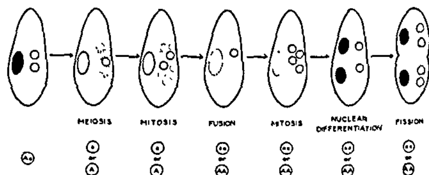


FIG. 20. Autogamy in a *Paramecium* heterozygous for the gene pair *A/a* (redrawn from Beadle, 1949, see Ref. 1).

animal, to begin with, was heterozygous for a given pair of alleles, say *A* and *a*, it becomes homozygous for one of the alleles after autogamy. Since the degeneration of nuclei is at random, the animals have equal chances to become homozygous for *A* or for *a*. In other words, a population of heterozygous *A/a* Paramecia contains, after autogamy, 50 per cent. of *AA* and 50 per cent. of *aa* animals.*

In the absence of conjugation, autogamy occurs periodically in the cultures. It can also be artificially induced.

You will have observed that the existence of these two modes of the sexual phenomenon offers great opportunities not only to the Paramecia, but to the geneticists as well. *Conjugation allows the transfer of a chosen gene from one cytoplasm into a different one, while autogamy allows the*

* The detailed description of the life-cycle of *Paramecium* and the relevant genetic evidence will be found in the review of Sonneborn (1947)[15]

substitution of one genotype for another one, within the cytoplasm of a given animal.

With these facts and rules in mind, we can now study the behaviour of a pair of alternative characters discovered by Sonneborn in 1943.[12] These two characters are called

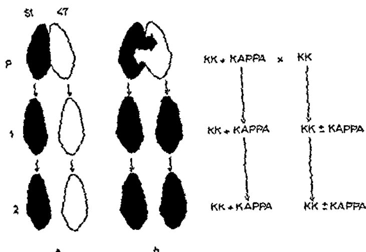


FIG. 21. Transmission of the killer (black) and sensitive (white) characters in a cross between Paramecia of races 51 and 47; *a*, without cytoplasmic exchange; *b*, with cytoplasmic exchange (after Sonneborn, 1949, see Ref 18).

respectively 'killer' and 'sensitive', and manifest themselves in the following manner. The culture medium in which a strain of killer Paramecia has grown contains a toxic substance, 'paramecin', which does not affect the killer animals, but kills Paramecia of other strains, classified as sensitives. The death of these animals, under the influence of paramecin, is preceded by characteristic morphological changes and is not rapid enough to prevent conjugation between two animals carrying the alternative characters.

The first cross we are going to study is between a killer animal of stock 51 and a sensitive one of stock 47 (Fig. 21). After conjugation has taken place and the exconjugants of one pair have fallen apart, each of them is isolated and

allowed to produce a clone by binary fission. These clones can now be tested. It is found that the clone formed by the killer exconjugant is killer, and that the clone formed by the sensitive exconjugant is sensitive. You remember of course, that, as a result of conjugation, the genotypes of the two exconjugants are identical. You know also that the genotypes of the two clones derived from the exconjugants by binary fission are identical with the genotypes of the exconjugants. It appears, therefore, that the characters of the two clones, *one killer, the other sensitive*, cannot be due to different nuclear constitutions, and this is confirmed by the observation of the second generation obtained by *induced autogamy. The character of all the animals* of each clone remains unchanged in spite of the fact that autogamy renders different animals of a heterozygous clone *homozygous* for different genes. The conclusion of these experiments is obvious: *the killer and sensitive characters are determined by the cytoplasm, rather than by the genes.*

The correctness of this conclusion is demonstrated by the study of abnormal conjugations. In normal conjugation, you remember, there is no exchange of cytoplasm between the conjugants. Sonneborn noticed, however, that some pairs of conjugants remain associated for an exceptionally long time and that sometimes one can see a rather wide cytoplasmic bridge between the two conjugants.[13, 14] It appeared to him that these abnormal conjugations might be accompanied by exchanges of cytoplasm. If so, would there be also an exchange of the cytoplasmic characters? Tests performed on animals derived from the killer and sensitive exconjugants did indeed show that, following these abnormal conjugations, the latter become killer: in other words, abnormal conjugation results in the 'contamination' of the sensitive conjugant by a parcel of killer cytoplasm. Further experiments showed that the killer character of the originally sensitive animals, thus acquired by cytoplasmic transfer, can become a permanent characteristic.



FIG. 22. *Paramecia* stained with Giemsa The animal on the left is a 'sensitive', the one on the right, a 'killer'. Deeply stained kappa-particles can be seen in the cytoplasm of the 'killer' (from Sonneborn, 1950, Ref. 19)

There is thus no doubt that the ability of killers to produce paramecin is linked to a peculiar property of their cytoplasm. A series of beautiful experiments performed by Sonneborn and his students soon showed that this property is due to the presence in the cytoplasm of killer animals of a discontinuous factor, that is, of particulate elements endowed with genetic continuity and distributed at random at fission. The size, of the order of 0·3 μ in diameter, and the average number (200–300) per animal of this factor could be calculated.[9, 10] The particles were christened *Kappa* and their appearance on the biological scene was greeted by universal cheers.

Kappa's popularity did not last however. The responsibility for this rests with one of Sonneborn's co-workers, Dr. Preer, who went a trifle too far, two years ago: he showed that kappa could actually be seen under the microscope in the cytoplasm of appropriately stained killer Paramecia.[11] The *Paramecium* shown on the right side of Fig. 22 is a killer. Its cytoplasm contains clearly visible, darkly stained kappa particles.* No such particles are visible in the cytoplasm of the animal on the left side of the picture, which is a sensitive.

The fact that kappa now became a visible material object was not the only reason, however, why it lost a great deal of its attractiveness: to put it that way would certainly be an exaggeration. For there was indeed what appeared to many as an even more serious circumstance: kappa could be stained with the Feulgen reagent, specific for desoxyribonucleic acids It is known that these substances are characteristic of chromosomes, on the one hand, and of certain viruses, on the other. It now appeared obvious to many biologists that kappa was a somewhat strange, but nevertheless vulgar virus-like parasite which, disguised as a normal cell constituent, sneaked on to the biological

* There are between 100 and 1,000 kappa particles per killer animal. The size of the particles, measured in stained preparations, is 0·2–0·5 μ diameter



FIG 22 *Paramecia* stained with Giemsa The animal on the left is a 'sensitive', *the one on the right, a 'killer'. Deeply stained kappa-particles* can be seen in the cytoplasm of the 'killer' (from Sonneborn, 1950, Ref. 19)

crosses appears, however, when a second generation is produced by autogamy. Contrary to what we observed earlier, in the cross now considered there is, following autogamy, a segregation within the clone formed by the killer exconjugant: half of the animals of this clone remain killers, the other half become sensitives. In the clone formed by the sensitive exconjugant, on the contrary, no segregation is observed under the same circumstances.

In order to account for these facts, the following interpretation was suggested by Sonneborn.[12] It is assumed that killer animals of stock 51 contain both kappa and a dominant nuclear gene, K, necessary for the multiplication and maintenance of kappa; but that animals of stock 32, on the contrary, carry neither kappa nor the dominant K: *instead their nuclei contain the recessive* k, in the presence of which kappa cannot multiply. The cross between a *killer of stock* 51 and a sensitive of stock 32, can then be represented as:

killer 51 *sensitive 32*
(KK+kappa) × (kk)

As a result of conjugation, the two exconjugants become heterozygous; their constitutions can be now written as:

killer *sensitive*
(Kk+kappa) and (Kk).

Although both exconjugants now carry the dominant gene K, only the first one is a killer since it contains both K and kappa. The second, on the contrary, *remains sensitive* in spite of the fact that it now has a similar genotype, for it contains no kappa. Thus kappa cannot be initiated by gene K, although K is necessary for its *maintenance*.

Following autogamy, the animals of each clone fall into two genotypes of equal frequency: KK and kk. But since Kappa is maintained only in the presence of K, we have, as a result of autogamy:

in the killer clone	*in the sensitive clone*
50% (KK+kappa)+50% (kk)	50% (KK)+50% (kk).

scene under Sonneborn's inadvertent watch. The enthusiasm now started cooling fast: kappa, by taking the wrong colour, obviously had trapped Sonneborn into the wrong discovery!

Science, as many of you know, has its fashions, and even scientists have moods. We shall not follow any of them. The question of the normal or parasitic nature of kappa

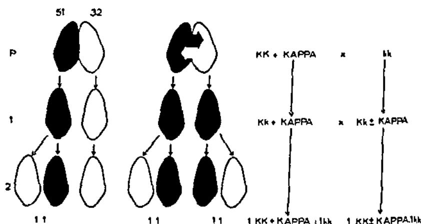


Fig. 23. Transmission of the killer (black) and sensitive (white) characters in a cross between Paramecia of races 51 and 32, with and without cytoplasmic exchange (modified after Sonneborn, 1949, see Ref. 18).

is an academic one, or, if you prefer, a question of phylogeny: the ultimate origin of kappa is hidden in the darkness of the past. However, whatever its ultimate origin, it provides us today with a model of a cytoplasmic particle responsible for a specific hereditary trait, and, as we will presently see, well integrated into the metabolism of killer Paramecia.

To show this we now turn to crosses between killers of the same stock, 51, with sensitive animals of a different stock, known as stock 32. This cross (Fig. 23) produces immediate (F_1) results entirely similar to those we observed earlier: the killer exconjugant gives rise to a clone of killers, the sensitive exconjugant forms a clone of sensitives. An important difference between the two

(*a*) It has been postulated that the F_1 killers are heterozygotes K/k. The cross of two F_1 killers should therefore produce killers and sensitives in a 3 : 1 ratio. (The cytoplasmic factor, kappa, does not intervene here, since both conjugants are killers.) This is what is actually observed. K is therefore dominant. The crossed animals are, as postulated, heterozygous.

(*b*) The exautogamous F_2 killers and sensitives derived from the killer exconjugant have been postulated to be homozygous respectively for K and k. Autogamy within these clones should therefore not modify their phenotypes. This expectation has been confirmed.

(*c*) It has been postulated that the F_1 sensitives have the same genotype as the F_1 killers (K/k). Therefore, the cross of an F_1 sensitive with an F_1 killer should give the following result: the descendants of the sensitive exconjugant, whatever their genotype, should all be sensitives since they contain no kappa; the progeny of the killer should, on the contrary, show a ratio of 3 killers : 1 sensitive (kappa multiplying only in the presence of K). This is in fact what happens. Experiment (*a*) having shown that the F_1 killers are heterozygotes K/k, it is clear that the F_1 sensitives possess the same genotype.

(*d*) Since the genotype of the F_1 sensitives is K/k, the F_2 produced by these animals by autogamy must comprise sensitives of the two genotypes K/K and k/k. If clones derived from exautogamous F_1 sensitives are crossed with F_1 killers (K/k+kappa), it would be expected that some of the crosses will involve a sensitive of genotype K/K. In such a case all the progeny of the sensitive should be sensitive, and all the progeny of the killer, killer. This expectation was fulfilled also, thus proving the occurrence of sensitives homozygous for K on the one hand, and, on the other, the incapacity of this gene, even when homozygous, to initiate the production of kappa.

(*e*) The relationship between the cytoplasmic factor, kappa, and gene K is further revealed by ingenious experiments devised to reintroduce the latter into sensitives derived by autogamy from F_1 killers. The cytoplasm of these sensitives is derived from that of killer exconjugants and their sensitive phenotype is ascribed to their nuclear constitution k/k. If the cytoplasmic property, which permits gene K to manifest itself, were transmitted in an entirely autonomous fashion, the reintroduction of K into this cytoplasm

Of these four classes, only the first one, which contains both kappa *and K, is a killer. The three others are* sensitives.

The interpretation offered by Sonneborn thus accounts, as you see, for all the observed results. Moreover, it is also compatible with the results of the cross between killers and sensitives of stocks 51 and 47 which I considered first. This cross, you remember, differs from the *one I have just discussed only in one respect: at autogamy* no segregation was observed among the animals of the clone produced by the killer exconjugant. All we have to assume now is that both the killers of stock 51 and the sensitives of stock 47 are homozygous for *K*. The killer phenotype here depends entirely on the presence or absence of kappa particles.

Sonneborn's interpretation permitted the formulation of a certain number of predictions which have been subjected to experimental proof and born out by experiments. Among these, one is particularly telling. Since it is assumed that, after conjugation between killers of stock 51 and sensitives of stock 32, each of the exconjugants becomes heterozygous for *K* and *k*, it was expected that the sensitive partner could be 'contaminated' in the course of conjugations involving a cytoplasmic transfer. The 'contaminated' animals should, of course, give rise to clones of killers. These killer clones should split, after autogamy, *into 50 per cent. of killers and 50 per cent. of* sensitives. Thus, after conjugation involving cytoplasmic transfer, a segregation should be observed in the clones derived from both exconjugants, although after normal conjugation it was observed only in the clone derived from the killer exconjugant. Fig. 23 shows that this expectation was fulfilled.

The correctness *of the general interpretation and, in particular,* of the genotypes ascribed to the different animals resulting from the cross killer 51 × sensitive 32 was verified by a number of experiments among which only a few will be quoted.

of the animals is increased to 3·4 by more abundant food, kappa's rate of multiplication is practically unchanged (2·0 doublings within 24 hours). This results in an increasing proportion of animals devoid of kappa particles.

The estimation of the proportion of animals which have lost kappa permitted Preer,[8] long before the discovery that kappa was microscopically visible, to compute the average number of particles per animal. On the other hand, the occurrence of such animals in the vegetative progeny of a killer was the earliest proof of the particulateness of the cytoplasmic factor involved in the manifestation of the killer phenotype.

Let us now, before we go on, stop a minute to compare the kappa particles of *Paramecium* with the cytoplasmic particles of the yeast cell. At first sight they differ profoundly in that kappa depends for its reproduction on a nuclear gene, while the cytoplasmic particles of yeast do not. This difference may, however, be due to purely fortuitous circumstances: one must remember that the demonstration of kappa's dependence on the nucleus hinged upon the accidental discovery of stock 32, which carries the recessive gene *k*. The independence with regard to the nucleus of the multiplication of the cytoplasmic factor of yeast may be purely apparent, and due to the fact that no gene mutation has thus far been detected which interferes with its maintenance. In turn, it is entirely possible that a mutant gene will be found some day in *Paramecium* which, similar to the recessive gene *r* of yeast, interferes with the production of paramecin even in the presence of kappa.

Another apparent difference between kappa and the cytoplasmic particles of yeasts is the ability of kappa to mutate to forms characterized by the production of different types of paramecin.[5] This difference may, however, also be but an apparent one, due to the fact that no adequate methods have been devised for the detection of mutations of the yeast cytoplasmic particles. It must be remembered, moreover, that there is no decisive evidence

should restore the killer phenotype. Gene *K* has been reintroduced into such sensitives by crossing them with killers. The results of such experiments depend on the time which has elapsed between the origin of the sensitive by autogamy of F_1 killers and the reintroduction of gene *K*. If the exautogamous animal has undergone during this time-interval two or three fissions, the descendants of the *k/k* exconjugants remain killers in most of the cases. If four or five fissions have intervened, the majority of animals become sensitives in spite of the reintroduction of *K*. Thus the cytoplasmic factor is rapidly lost in the absence of gene *K*.

Let us sum up. *The killer phenotype offers us another example of a character, the development of which depends both on the cytoplasm and on the nucleus. In order to be established and maintained, it requires the simultaneous presence of an autonomous cytoplasmic particle, kappa, and of a dominant nuclear gene, K. Gene K is*, as we have seen, *incapable of initiating the production of kappa, but is necessary for its continued maintenance.* To this I should like to add a brief mention of the recent discovery that, under a given set of conditions, the average number of kappa particles per animal is proportional to the dosage of *K* genes: homozygous *K/K* animals contain twice as many kappa particles as heterozygous *K/k* animals.[3]

The autonomy of kappa is therefore only relative; but it is considerable. This is shown by the fact that when external conditions (such as the food supply or the temperature) or the physiological state of the cells (such as those produced by ageing or by the intervention of sexual phenomena) are modified, the average number of kappa particles per animal varies: it can decrease or increase. The rhythms of multiplication of kappa and of the animals are not necessarily synchronous.[8, 9, 10, 14, 16]

In one strain of killer Paramecia, for example, it could be shown that the relative rates of multiplication of kappa and of the animals change as a result of modified food supply.[8,9,10] When killer animals of this strain undergo 2·6 fissions per day, kappa undergoes 2·1 doublings during the same time interval. When the rate of fission

of these antibodies is detected by their action on individuals of the same strain. Normally Paramecia are lively swimming animals; in the presence of minute amounts of specific antibody they are rapidly immobilized. Apparently their cilia carry the antigen. In the presence of antibody the cilia stick together and can no longer beat in the co-ordinated way required for propulsion.

Sonneborn has shown that, if a series of independent cultures of Paramecia are compared in this manner, they most frequently exhibit different antigenic traits. If, for example, three antisera are prepared by injection into rabbits of three strains of Paramecia, A, B, C, each of the antisera specifically inhibits animals of the corresponding strain, that is, anti-A serum inhibits animals A, anti-B inhibits B, and anti-C inhibits C. Cross reactions occur very seldom.

Although each antigenic type is stable both in vegetative and sexual reproduction under a given set of conditions, it does change occasionally. Moreover, Sonneborn and Beale have shown that changes from one antigenic type to another can be experimentally induced. Thus, different lines of Paramecia, *all derived from a single homozygous individual*, can exhibit many different antigenic properties. However, of all the antigens which an animal is potentially capable of producing, only one is produced at a time. Fig. 24 illustrates these facts.

The role of the nucleus and of the cytoplasm in the determination of the different antigenic traits has been studied by Sonneborn and his co-workers in different varieties and stocks of Paramecia. It can easily be shown that these traits depend on both the cytoplasm and the nucleus: for this purpose essentially the same techniques are used as those employed in the study of the killer character. The study of the results of conjugation between animals of the same race and stock, but of different antigenic types, say A and B, shows that the antigenic type is controlled by the cytoplasm: unless there occurs exchange

thus far that the vegetative mutants of yeast are the result of the loss, rather than of the mutation of the cytoplasmic particles to an inactive form (see above, p. 33).

But even granting the present differences between kappa and the cytoplasmic factor of yeast, there are very important similarities between these elements, notably their particulate nature (well established in the case of kappa, highly probable in the case of the yeast) and the irreversible character of their loss. In this respect they belong to the same class of entities as the plastids and other structural elements briefly mentioned earlier, and distinctly differ from the cytoplasmic conditions which I shall consider now.

2. *INHERITANCE OF ANTIGENIC TYPES IN PARAMECIUM; DAUERMODIFICATIONEN* AND MATING TYPES

The discovery of these new cytoplasmic conditions is another, more recent feat of the fertile activity of Sonneborn and his co-workers. To those discouraged by the possible ultimately parasitic origin of kappa this must have been cheering news: here, at last, were cytoplasmic properties hardly ascribable to the intrusion of a parasite, and what is more, properties determined by something in the cytoplasm which will probably never be seen (unless, of course, one looks too close; but then one can see even molecules, for they are particles too!) At last, Sonneborn had redeemed his sins!

The new cytoplasmic conditions I am referring to control the serological properties of Paramecia which can be revealed by the now classical methods of immunology.[22, 21] When a culture of Paramecia is repeatedly injected into rabbits, there appear, in the blood of the injected animals, antibodies which are specifically directed against the antigens of the injected strain. The presence

obvious when crosses are made between animals of different stocks. Such stocks may differ in the array of antigens which the animals can produce. For example, animals of one stock can alternatively produce antigens A, B, C, D. Animals of another stock also can produce antigens A, B, C, D, but, in addition, they can produce antigen F. This difference is gene controlled, for conjugation between animals of the two races, which equalizes their genotypes, also equalizes their capacities to produce F.

The respective functions of the nucleus and the cytoplasm in the determination of antigenic types, which I have just outlined, will appear to you in a more precise way when we follow through a series of experiments performed by Beale on races 60 and 90 of variety I of *Paramecium aurelia*.[2] To begin with, in each of these two races a single individual is taken which has just undergone autogamy: each is therefore homozygous for all its genes. All experiments will be performed with cultures derived from these two individuals.

A first experiment consists simply in placing some animals of each of the two cultures at three different temperatures, 18°, 25°, and 29°, and in allowing them to multiply for several days. Some animals of each culture are then injected into rabbits: six antisera are thus prepared. They can now be tested on the remainder of each culture

Let us call the animals raised at 18°, 25°, and 29°, S, G, and D respectively, and let us first see what the reactions are between the antisera obtained by injection of animals of race 60 and the homologous animals. Table II shows in its upper left corner that each type of animal is paralysed only by homologous antiserum, that is, by the antiserum obtained after injection of animals of the same culture. Thus, animals S are paralysed only by anti-S serum, animals G only by anti-G serum, and animals D only by anti-D serum It appears that animals of the same

of cytoplasm, the clones formed by each of the conjugants (*which, you remember, after conjugation are genotypically* identical) exhibit the same phenotype as that of the animal

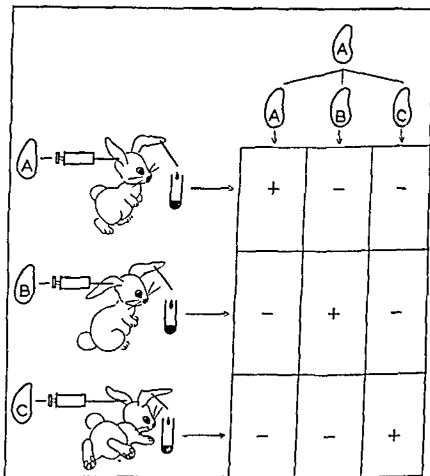


FIG. 24. Diagram showing the interaction between Paramecia of three different clones derived from a single animal and antisera prepared by injection into rabbits of the same animals. + indicates paralysis of the animals in the presence of antiserum, —, its absence (modified after Sonneborn, 1950, see Ref. 20).

from which it was derived. When cytoplasmic exchange does take place, the antigenic type of one of the exconjugants is changed into that of the partner.

However, the nucleus also plays a role: this becomes

You will wonder, of course, why similar symbols are used to designate antigens which have proved to be different. The answer to this is that, as you will see in a moment, antigens D and G of races 60 and 90, in spite of the differences just revealed, are homologous to each other.

This will appear clearly when we examine the results of some experiments designed to answer the following question: granted that homozygous animals are capable of exhibiting an array of antigenic phenotypes, and that animals belonging to different stocks differ in the 'constellation' of antigens which they are capable of producing, what factors control the nature and limits of this potential of variation? Are they nuclear or cytoplasmic?

I should like to emphasize that the question I am raising concerns the *capacities* of Paramecia to exhibit a certain number of antigenic phenotypes, and not the phenotypes themselves.

In order to answer this question, Beale crosses animals of races 60 and 90 raised at 25°, for example, that is 60 G and 90 G. The results of this cross can be followed on Fig. 25. After the partners have separated, they are isolated and allowed to undergo four or five fissions. The clones thus formed by each of the exconjugants are tested for antigenic type. It is then found that all animals of each of the clones carry simultaneously antigens 60 G and 90 G. These animals can now be induced to undergo autogamy. Tests of the exautogamous animals show that, as a result of autogamy, one-half of the animals of each of the clones becomes 60 G, the other half 90 G.

These results are best explained by assuming that the two animals which were crossed (60 G and 90 G) differed in one pair of Mendelian genes. As a result of conjugation each of them became heterozygous for the alleles carried by each of the parents: hence they exhibited both antigens 60 G and 90 G. At autogamy, the two alleles segregated in each of the clones: thus, 50 per cent. of the animals in

stock raised at different temperatures develop different antigenic types, which we can call (like the animals) S, D, and G. Only one of these antigens is exhibited at a time.

TABLE II

	Serum anti-					
Animals	60 S	60 D	60 G	90 S	90 D	90 G
60 S	+	–	–	+	–	–
60 D	–	+	–	–	–	–
60 G	–	–	+	–	–	–
90 S	+	–	–	+	–	–
90 D	–	–	–	–	+	–
90 G	–	–	–	–	–	+

The same comparison can be made between S, D, and G of race 90: the same relationships are observed (Table II, lower right). Here again we find that each of the antisera paralyses only the homologous animals. It is clear that animals of stock 90 also exhibit, according to the temperature at which they were raised, three different antigenic types: S, G, and D.

One may wonder legitimately, however, whether the antigens called S, D, and G are identical in the two races. The answer to this question is given by the reactions recorded in the lower left and upper right squares of the same Table II. It can be seen that S animals of either stock are indiscriminately paralysed by both anti-S sera. S antigens of races 60 and 90 seem therefore to be identical. The situation is, however, different with respect to D and G. Here animals of each of the cultures are paralysed exclusively by the homologous antiserum. Animals 60 D, for example, are paralysed only by anti-60 D serum, not by anti-90 D serum. It appears, therefore, that by and large each of the stocks forms a series of antigens of its own. Clearly, antigens D and G of race 60 are different from antigens D and G of race 90.

further the genotypes of the exautogamous animals which, you remember, are of the two types 60 G and 90 G. In order to do this, let us place them now at 29°. After they have undergone a few fissions, let us again determine their antigenic type: this should show us how autogamy has affected their capacities to form antigen D. The result is most interesting indeed: we find that, among the clones which were, to begin with, 90 G, some produce, when raised at 29°, not the 90 D, but the 60 D antigen. Conversely, among the 60 G animals, some produce the 90 D and not the 60 D antigen. In other words, all four possible combinations of the two types of G and D are found (60 G–60 D; 60 G–90 D; 90 G–60 D; 90 G–90 D). Clearly, autogamy results in the recombination of antigenic traits, just as it results in the recombination of Mendelian genes.

These results indubitably provide an answer to the question we raised. *The capacities to produce each one of a certain array of antigenic types*—say D, G, and S—*depend on the presence of an equal number of non-allelic genes.* Each of the two races studied carries these three genes; but at two of the three loci (d and g) they are represented by different alleles. In race 60 we may call these genes: d^{60} and g^{60}; in race 90: d^{90} and g^{90}. The genotypes of the animals of races 60 and 90 can then by symbolized by the formulae given in Table III.

TABLE III

Race	*Antigen exhibited*			*Genotype*
	29°	25°	18°	
90	90 D	90 G	S	$d^{90}g^{90}s$
60	60 D	60 G	S	$d^{60}g^{60}s$

It must be emphasized again at this point that, although each of the animals of each race is potentially capable of exhibiting a number of different antigens, it effectively exhibits only one at a time. And this leads us to a second

each clone became 90 G, and 50 per cent. 60 G. The observed behaviour of the two antigenic characters 60 G and 90 G in crosses is thus satisfactorily accounted for.

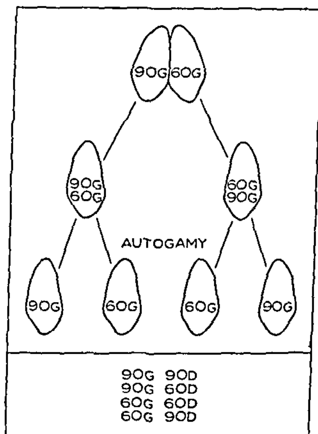


FIG. 25. Transmission of antigenic type G in a cross of Paramecia of races 90 and 60 Below are given the four combinations of the *capacities* to form antigens G and D observed in the F_2

Similar crosses have been performed between animals of races 60 and 90 raised at 29°, that is between animals of antigenic types 60 D and 90 D. These crosses give results identical with those I have just described. Thus, here again, a *similar conclusion is reached: animals of types 60 D and 90 D behave as if they differed in one pair of* Mendelian genes.

But let us return to the first cross and let us explore

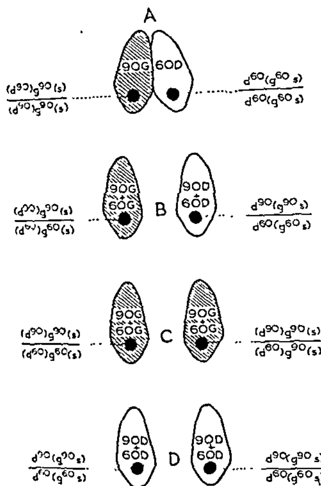


FIG 26 Cross between Paramecia of antigenic types 90 G and 60 D. The immediate results of the cross (A) are shown in B. C and D illustrate the transformation of type undergone by the clones derived from the exconjugants when grown at 25° and 29° respectively. In the genotypic formulae of the different animals, the genes which are not manifested are enclosed in brackets (redrawn from Beale, 1952, see Ref. 2)

question which can be stated as follows: granted the existence of a certain number of gene-controlled capacities to form a variety of antigens, what determines which of these capacities will be effectively elicited?

Before I describe the experiments which answer this question, I must add the following information. As you have seen, *it is possible, by raising the progeny of a single* homozygous individual at three different temperatures, to establish clones of three different antigenic types. These changes of antigenic type are reversible: a clone raised at 29°, for example, and exhibiting antigenic type D, will, when placed at 25°, be converted into antigenic type G. Thus, *all* three described antigenic types, D, G, and S, are interconvertible and can be changed into each other according to the temperature. These changes are not immediate, however: if, for instance, animals of type D are placed at 25°, the conversion into G will be accomplished only after some fifty fissions. In the meantime the animals remain type D. The 'switch' then intervenes in a sudden manner: all animals are transformed in the course of two fissions. The existence of this 'latent period' makes it possible to cross individuals of two different antigenic types and to establish the immediate effects of conjugation on the exconjugants themselves and on their immediate vegetative progeny. This will permit us now to answer the second question we raised.

The cross we are going to study is between animals 90 G and 60 D (Fig. 26 A). After conjugation is accomplished, the two exconjugants are isolated and allowed to undergo four or five fissions. The characteristics of the two clones thus formed can now be determined. We find that the clone derived from exconjugant G carries nothing but G antigens, but these are a mixture of 60 G and 90 G. Similarly, the progeny of exconjugant D carry only D antigens, but these again are of both the 60 D and 90 D types. Since the genotypes of the two exconjugants are identical after conjugation, it is clear that it is not the

in this respect that the cytoplasmic states determining the antigenic types of Paramecia differ so radically from the cytoplasmic properties we studied earlier, and it is this aspect which imposes a different interpretation.[19, 21]

Each of the antigenic types might, of course, be ascribed to the presence in the cytoplasm of a different type of mutually exclusive, alternative cytoplasmic particle or plasmagene, autonomous in its reproduction, but exhibiting a specificity dictated by the nuclear genes. The predominance, under a given set of conditions, of one type of plasmagenes, to the exclusion of the others, could be explained by a sort of intracellular competition or selection. However, the fact that these plasmagenes are apparently never definitively lost, does introduce a great difficulty, which is reinforced by the fact that, in crosses between individuals of two races differing in the spectrum of antigens which they can produce, the ability to develop a certain antigen appears to be correlated, in the absence of cytoplasmic exchange, with the distribution of genes. The difficulty can be alleviated by assuming that these plasmagenes, by contrast to the particles we have met with earlier, can be *initiated* by the genes; but if we do so, we must go further and question the usefulness of invoking particles altogether, for, as Delbruck[4] pointed out, the occurrence of alternative cytoplasmic states endowed with diverse degrees of stability can be explained without it.

Imagine that the cell is the seat of two reaction-chains, respectively converting substrates a_1 and b_1 first, with the help of enzymes A_1 and B_1, into intermediates a_2 and b_2, then, with the help of enzymes A_2 and B_2, into the final reaction products a_3 and b_3 (Fig. 27). Under constant external conditions such a cell will soon reach a stable equilibrium, characterized by a certain concentration of the intermediates a_2 and b_2.

The situation will be very different, however, if there is an interaction between the two sets of reactions. Assume, for example, that, when the intermediate a_2 reaches a

nucleus, but the cytoplasm which is responsible for the antigenic type elicited in the hybrid: in other words, it is the cytoplasm of each of the exconjugants which determines which of the genes will manifest itself. Fig. 26 B shows that in the exconjugant on the left, the cytoplasm of which was conditioned at 25°, the *g* genes come into action; and that in the exconjugant on the right, raised at 29°, the *d* genes manifest themselves.

Some animals of each of the clones can now be placed at 25°, some others at 29°. After they have multiplied, their phenotypes can be determined again. Fig. 26 C shows that, at 25°, the progeny of the exconjugant on the right changes its antigenic phenotype, while at 29° it is the progeny of the exconjugant on the left which undergoes transformation (Fig. 26 D). It is evident that the cytoplasm of the 90 G animal, raised at 25°, possesses the property of calling into action the genes at the *g* locus (whether this locus is represented by the allelomorph g^{90} or g^{60}), while the cytoplasm of the 60 D animal, differentiated at 29°, calls into action the genes of the *d* locus (that is d^{60} or d^{90}). *This property of the cytoplasm of permitting the expression of one particular gene out of the many present, is,* as we have seen, *persistent, even though it is reversible.*

Summing up, in *Paramecium*, the establishment of definite antigenic phenotypes depends on the action of distinct nuclear genes. It is, however, the cytoplasm which, depending on the external conditions, calls into action one of a series of non-allelomorphic genes. The external conditions, such as temperature, determine which of the several possible interconvertible but mutually exclusive states the cytoplasm will assume. Each of these cytoplasmic states is stable under a given set of external conditions. Changes in the external conditions result in changes of the state of the cytoplasm, but the latter presents a certain inertia: it tends to perpetuate itself. However, in spite of this tendency, *none of the cytoplasmic states can be definitively stabilized* so long as the genotype remains unaltered. It is

cytoplasmic control of mating types in the varieties of Paramecia belonging to the so-called group B of varieties. Variety 4, which comprises the two mating types VII and VIII, may be taken as example. According to Sonneborn,[17] and Sonneborn and Dippel,[23] any one clone of variety 4 is usually pure for either mating type VII or VIII, although neither of these reproduces absolutely true to type. Conjugation, although it results in identical genotypes of the two exconjugants, does or does not equalize the mating types of the mates, depending on whether it is accompanied by cytoplasmic exchange or not. In the absence of cytoplasmic exchange, the mating types of the conjugants do not change, and each of the exconjugants gives rise to a clone of the mating type characteristic of the 'cytoplasmic parent'. When exchange of cytoplasm does occur, both exconjugants most frequently produce identical type VIII clones. More rarely the clones formed by both exconjugants are type VII, or mixed, each of them containing both type VII and type VIII lines.

Dauermodificationen.[6, 7] Jollos described many years ago long-lasting and presumably cytoplasmic changes (*Dauermodificationen*) induced in Paramecia by heat-treatment and various chemicals. These modifications are propagated over long periods of vegetative reproduction in the absence of the inducing agent, but most frequently disappear at the time, if not in the course, of sexual reproduction.

As pointed out by Sonneborn,[11, 15, 21] *Dauermodificationen* are in many ways similar to the changes of antigenic types described above. Obviously they suggest similar interpretations.*

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* In my opinion, precise information concerning the fate of the old macronuclei in conjugation and autogamy is the only missing element of importance in the picture presented in this chapter.

certain concentration, it inhibits the reaction leading to the formation of b_2. Make a similar assumption concerning the action of b_2 on the production of a_2. We have then a system which, under the influence of *transitory changes* in the external medium (such as changes in the relative

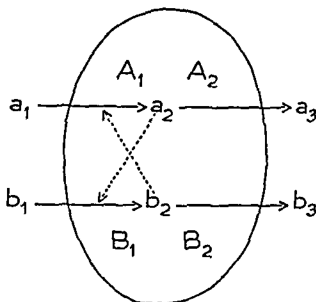


FIG. 27. Delbrück's model of the establishment of alternative cytoplasmic metabolic states (redrawn from Delbrück, 1949, see Ref. 4).

supply of the two substances), will assume two alternative states of flux equilibria, characterized by the functioning of either one or the other of the possible reactions. Each of these states, *established without any change in the intrinsic constitution of the cell,* will be endowed with a great stability, which may give us the impression of permanence, that is, of inheritance; yet they will be reversible precisely because the changes of state do not involve changes in constitution.

A model of this sort can certainly be taken as a basis of interpretation of the alternative antigenic types of Paramecia.

Mating types. The same dilemma—competing plasmagenes or alternative steady states—presents itself in the consideration of the

III

'Lorsque certains effets révèlent une certaine dissymétrie, cette dissymétrie doit se retrouver dans les causes qui lui ont donné naissance.'

(PIERRE CURIE, Sur la symétrie dans les phénomènes physiques, *J. Phys.* 1894, 3, 393–417)

1. THE PHENOMENON OF 'BARRAGE' IN *PODOSPORA ANSERINA*; CYTOPLASMIC INHERITANCE IN OTHER FUNGI

THE last case I am going to consider in some detail is that of a mould, *Podospora anserina*, which taxonomists, in order to make things simpler no doubt, also call sometimes *Pleurage anserina* or *Sordaria anserina*. This organism is a close relative of the famous *Neurospora* which, thanks to the brilliant studies in biochemical genetics performed on it since 1941 by American workers under the leadership of Beadle, is known today even to most biochemists. *Podospora* is not known in the same circles. It is familiar to horse-lovers rather than to biochemists, for its mycelia can be frequently found growing on horse-dung. Although during the last ten years it has been the object of beautiful genetic studies by my friend Rizet, it is not well known to geneticists either, for Rizet's publications were much delayed by the war and the occupation of France. Thus the war has interfered with the career of *Podospora*, just as it has with the careers of so many of us.

First, let me say a few words about the life-cycle of *Podospora*,[29] schematically represented in Fig. 28. The mycelium shown on the left side of the figure is, strictly speaking, acellular: it is subdivided into a certain number of compartments, but the partitions separating these compartments are incomplete and presumably allow the migration of nuclei from one compartment to the next one. In any case, the number of nuclei per compartment is

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contains two nuclei of opposite mating type and which will give rise to the asci, about 100 per perithecium. Within each ascus, the two nuclei fuse. Meiosis, consisting of the two usual nuclear divisions and accompanied by crossing over, then takes place, and is followed by a third, equational division, thus giving rise to eight nuclei in each ascus. Spore formation now follows. Each spore encloses two non-sister nuclei. Fig. 29 shows how the orientation of the spindles during the three nuclear divisions achieves this result. It shows also that the distribution of the mating type genes, mt^+ (white) and mt^- (black), depends on the intervention of crossing over. If crossing over occurs, there is *postreduction* of mating type genes; each of the four spores of the ascus contain one mt^+ and one mt^- nucleus. Such an ascus is called a *type I ascus*. If there is no crossing over, there is *prereduction*: two spores of the ascus each contain two mt^+ nuclei, and two other spores each two mt^- nuclei: they are *type II asci*. With respect to mating type, 98 per cent. of the asci are of type I.

Apart from binucleate spores, one sometimes finds in the asci smaller spores, containing only one nucleus. Such spores occur in pairs: one pair of uninucleate spores obviously replaces one binucleate spore. Therefore, in asci of type I, two uninucleate spores of any one pair carry each a different mating type gene, one mt^+ and one mt^-, while in asci of type II they carry identical genes: either mt^+ or mt^-.

When the spores are extracted from the ascus and placed on the surface of an appropriate medium, they germinate and give rise to new mycelia. Binucleate spores from type I asci, which carry both mating type alleles, give rise to fertile mycelia. On the other hand, binucleate spores from type II asci, and uninucleate spores, give rise to mycelia containing only one of the mating type genes and are therefore sterile.

If, instead of mating type genes, we now consider some other pair of genes, say P and p, which affect the size and

variable. The nuclei are haploid and belong either to a single, or to two or more different genotypes. When they are all of one sort, the organism is said to be *homocaryotic*; when they are of different sorts, it is called *heterocaryotic*.

With respect to mating type, homocaryotic strains are either + or −. In either case they are self-sterile: each

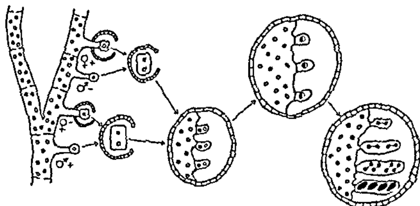


FIG. 28. The life-cycle of *Podospora anserina*. (Explanation in text.) (Courtesy of Rizet, unpublished.)

can form both male and female sex-organs, but fertilization does not take place. This makes the difference between sex and mating type very clear: mating types represent a mechanism not of sex, but of autosterility. Heterocaryotic strains which, like the one shown in Fig. 28, contain nuclei, or rather genes, of both mating types are, on the contrary, fertile. Here the male elements, which are microscopic uninucleate cells called *microconidia*, play the role of spermatozoa. They belong either to the + or the − mating type and can fertilize the female elements, the *ascogones*, which also are of both mating types and uninucleate. However, fertilization can occur only between elements of opposite mating type. Following fertilization, the two nuclei multiply within a cytoplasmic mass enclosed in a purely maternal envelope: the entire structure is called a *perithecium*. This multiplication is followed by the separation of small masses of cytoplasm, each of which

shape of the spores (Fig. 30), we find essentially the same behaviour; that is, the same two types of asci are present and only the relative frequencies of the two types are

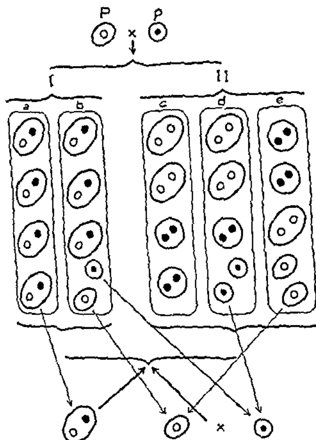


FIG 30 Cross between two strains of *Podospora anserina* each derived from a mononucleate spore and differing in one pair of mendelian genes, P and p Read downwards, the figure gives the results observed in the F_1, and read upwards, the results of further generations (from Rizet, 1952, see Ref. 19)

different. Here again all normal spores in type I asci contain two sorts of nuclei, P and p: they are big and oval, for they exhibit the dominant character P. In these same asci, when two uninucleate spores are present, one of the

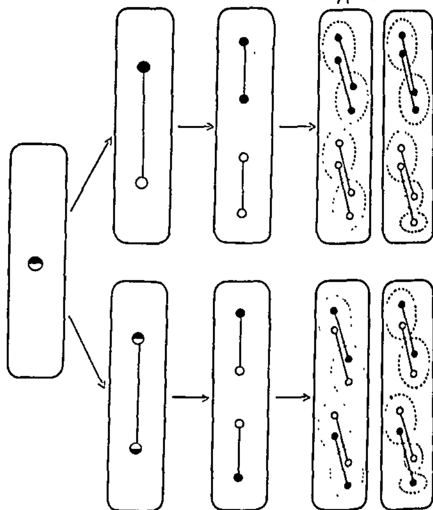


Fig. 29. Diagram illustrating how the orientation of spindles during spore formation in *Podospora* leads to the formation of two different types of asci, depending on the occurrence or non-occurrence of crossing over (redrawn from Rizet and Engelman, 1949, see Ref 20)

pair is *P* (oval), the other *p* (round). Type II asci contain two big oval and two smaller, roundish spores; the former contain two *P* nuclei, the latter two *p* nuclei. A pair of uninucleate spores in these asci are either both *P* or both *p*.

The mycelia being the product of spore germination, you can see that homocaryotic strains originate from uninucleate spores. Heterocaryotic strains, on the other hand, originate from binucleate spores.*

At first sight, one might think that binucleate spores from type II asci should give rise to homocaryotic mycelia. It must be remembered, however, that, because of the independent segregation of non-allelic genes, an ascus which is of type II with respect to one gene is most of the time of type I with respect to another gene. In practice therefore binucleate spores never give rise to truly homocaryotic strains.

This is all I am going to ask you to remember about the life-cycle of *Podospora*. I am afraid I have bored you in spite of the fact that I have shortened the story by leaving out some of its obscure parts: but all this was really necessary to enable you to understand the most interesting phenomena[19] which are observed when different strains are 'confronted', that is, are permitted to establish contact with each other as a result of their growth on the same medium. Under such conditions the behaviour of the mycelia permits them to be classified into two categories, which we will call *S* and *s*.

Let us inoculate, on the surface of the same nutritive medium, at a distance of, say, 5 cm. apart, two uninucleate spores or two small fragments of mycelia of two strains, both of which are either *S* or *s*. In any of these cases mycelia grow out and extend by radial growth until they meet. Growth then slows down considerably, but it does not stop at once and one can observe that, prior to the arrest of growth, the mycelia grow into each other over a distance of a few millimetres and, on the meeting line,

* Possibly also from the vegetative fusion of hyphae of two different mycelia.

the hyphae of the two strains frequently form anastomoses. The older and thicker parts of the mycelium are pigmented; the zone of anastomoses is usually thin: the meeting line therefore appears to the eye as a more transparent zone (Fig. 31, left side). When the two strains are of the same mating type, this is all one can see. When they are of opposite mating type, the meeting line is grossly marked by the development of a rather narrow row of darkly pigmented perithecia, that is of fruiting bodies resulting from cross-fertilization. This can be seen in the Petri dish represented on the left of Fig. 32, in which an *S* ascospore was planted in the middle and four others around it. Contacts between the five mycelia are marked by single lines.

Things are very different, however, when, of the two confronted strains, one is *S*, and the other *s* (Fig. 31, right side). In this case an apparently mutual inhibition manifests itself when the two strains come near each other. In the meeting zone, 1 to 2 mm. wide, the growth of the hyphae becomes disorganized. They do not anastomose, but branch and form a thick network. The mycelia are deeply pigmented, except in or near this zone, which thus appears to the eye as a transparent space 3 to 4 mm. wide. If the two strains are of opposite mating type, deeply pigmented perithecia are formed on each side of this space, or '*barrage*', which is thus clearly marked by a double instead of a single row of fruiting bodies. This can be seen on the right of Fig. 32: in this case an *S* ascospore was planted in the middle and four *s* spores around it.

The Frontispiece shows the results of a simultaneous confrontation of an *S* mycelium with mycelia of the two sorts.

As you can see, the '*barrage*' is a special and characteristic type of antagonism which does not interfere with the sexual process: an antagonism which is superimposed, one might say, *on* the system of auto-incompatibility realized by the mating type system.



FIG 31 Photograph illustrating the 'barrage' phenomenon. In the Petri dish on the left two *S* strains are confronted; the contact line between the two strains is a simple thin transparent line. The same is observed when the two confronted strains are *s* The Petri dish on the right shows the results of the confrontation of *S* with *s* Here a barrage, i.e a transparent zone of mutual inhibition, can be observed between the confronted strains (courtesy of Rizet, unpublished)

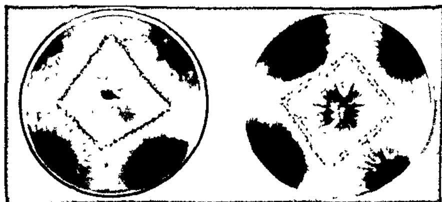


FIG 32 Photographs of two Petri dishes illustrating the 'barrage' phenomenon in *Podospora anserina* In the Petri dish on the left an *S* strain was inoculated in the middle and four other *S* strains, of opposite mating type, around it The contact line is indicated by a single row of fruiting bodies (perithecia). In the dish on the right, the culture in the middle is *S*, the cultures around it *s*, of opposite mating type The barrage is clearly marked by a double row of perithecia (from Rizet, 1952, Ref 19)

as a result of a cross between an *S* and an *s* strain, each derived from a uninucleate spore in order to be sure that the strains are homocaryotic. The left part of the figure, which we shall examine first, shows the results of confrontations of the spore progeny with a standard *s* strain. You can see that two types of asci are found: in one, all four spores give rise to mycelia which form 'barrages' when confronted with *s* mycelia; they are symbolized in the figure by a double bar. In the other type of asci, two spores show a similar behaviour; the other two give rise to mycelia which do not form a barrage with *s* mycelia. These results leave no doubt that the two sorts of strains differ in one gene pair, *S* and *s*, and find their explanation in purely Mendelian terms if it is remembered that the spores are binucleate, and if it is assumed that *S* is dominant over *s*. All spores of type I asci contain one *S* and one *s* gene. They therefore all manifest the character *S*, that is form barrages with *s*. In type II asci two spores contain two *S* nuclei and manifest the same character, two others contain two *s* nuclei and therefore give rise to mycelia which form no 'barrage' in the confrontation with the same standard *s* strain.

If this interpretation were entirely correct, confrontations of the same spore progeny with *S* should give a strictly reverse picture. What actually is observed is shown on the right side of the same Fig. 33. The spores of both types of asci which we classified as *S*, again come out as *S*: they do not form barrages with the standard *S* strain. However, the two spores in the type II asci which we supposed to be *s*, because they did not form a barrage with the standard *s* strain, do not form barrages with the standard *S* strain either. *In other words, s spores do not reappear in the asci as they should, and, instead, we find the equivalent number of spores of a new type, which are neither S, nor s, and which will therefore be called 'modified s'* and symbolized as s^s. This conclusion, drawn from the two sets of confrontations, is illustrated in Fig. 34, where the

Both *S* and *s* strains are perfectly stable in the course of vegetative reproduction, and can therefore be conveniently crossed. Crosses are performed best by spreading micro-conidia of one mating type on a mycelium of opposite

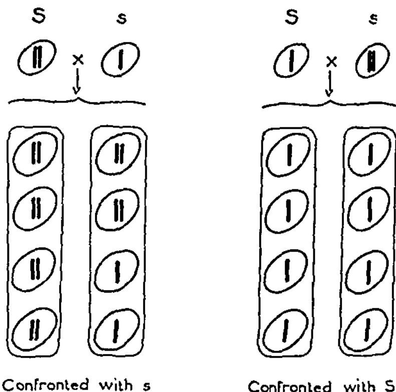


FIG. 33. Cross $S \times s$. Results of the confrontations with standard *S* and *s* of the mycelia produced by the spore progeny of the cross. A double bar stands for 'barrage', and a single bar for 'no barrage'.

mating type. The characteristics of the progeny of such a cross, that is of the mycelia formed by each of the spores, can be tested by confronting these mycelia with standard *S* and *s* strains. It is then found that, *regardless of the direction of the cross* (that is whether the maternal parent is *s* or *S*), the *s* character undergoes a very remarkable change in the course of the cross, while other genetic markers show a perfectly normal Mendelian behaviour. Fig. 33 gives the results of such determinations performed on asci formed

new type of spores, s^S, do carry s genes, but that the characteristic manifestation of these genes is somehow 'masked' as a result of the cross. This interpretation is borne out by the study of pairs of uninucleate spores found in the asci under consideration (Fig. 34, second, fourth, and fifth asci). The two members of a pair of spores extracted from type I asci are found to have different characters: one is S, the other s^S. It is clear therefore that the binucleate spores of these asci contain indeed two different nuclei. The members of a pair of uninucleate spores from type II asci possess, on the contrary, the same characters: they are both either S, or s^S. As expected, they contain identical nuclei.

The interpretation is borne out also by the results of further generations of crosses: the F_2, F_3, &c. . . . (Fig. 34, read upwards). I will not spend any time on presenting these results and will only mention that a binucleate spore of a type I ascus, postulated to contain one S and one s nucleus, indeed gives rise to a mycelium, the fruiting bodies of which again contain the two types of asci, thus proving that it did contain two different nuclei.

What kind of mechanism is responsible for the fact that the recessive character s does not emerge as such from the cross? When one turns to consider this question, several somewhat analogous cases come to mind, which have long since been described under the general title of *predeterminations*: one of the most illuminating studies in this field is that of Mather on heterostyly in plants. Formally, 'predetermination' simply means that the phenotype of an organism corresponds not to its own genotype, but to that of the unreduced ovum. Take, for example, an ovocyte heterozygous for a gene pair, say A/a, and suppose that, after meiosis, it became a. Fertilized by a spermatozoon carrying a, it gives rise to an organism of genotype aa, which should exhibit, and usually does exhibit, the recessive trait a. In cases of predetermination, however, its phenotype is characteristic of the dominant A, which

phenotypes are marked in each spore. (Consider at present only the first and third asci from the left, which contain each four spores.)

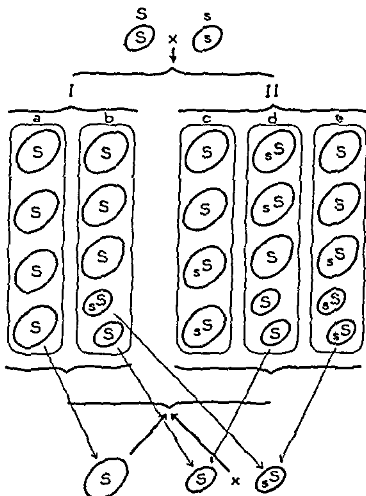


FIG. 34. Cross $S \times s$. Phenotypes as determined by testing the spore progeny in the manner indicated in Fig. 33 Read downwards, the figure gives the result of the F_1; read upwards, the results of further generations.

Compare with Fig. 30, which shows the behaviour of a purely Mendelian character (from Rizet, 1952, see Ref. 19).

In order to reconcile these results with a regular Mendelian mechanism of gene distribution, the operation of which is indubitably indicated by the proportion and distribution of the two characters, we must assume that the

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was present in the ovocyte, but is no longer present in the genotype of the egg. This persistent influence of the eliminated dominant is usually ascribed to, and in some cases has been proved to be due to, the presence in the cytoplasm of the egg of specific substances elaborated before meiosis by the dominant gene *A* and persisting after its elimination.

The disappearance of the *s* character of *Podospora* and the appearance in its stead of character *modified s* could, at first sight, be ascribed to a similar mechanism. One might assume that some sort of substance, elaborated in the hybrid mother cells of the asci by the dominant *S*, persists in the spores containing only *s* nuclei after meiosis, and confers to the mycelia which grow out of the spores their new *modified s* (s^S) characteristic. However, if this interpretation were correct, the hypothetical substance should be rapidly diluted in the mass of protoplasm resulting from subsequent growth, and the strain should therefore rather rapidly recover the *s* property which corresponds to its supposedly unchanged *s* genotype.

This corollary of the predetermination hypothesis can be tested in two ways. One can either subject the *modified s* strain to prolonged vegetative growth by serial transfers, where each new culture is started from a small piece of mycelium of the old one; or one can perform a series of crosses between two s^S strains: here, each new culture is started from the microscopic single ascospore. The dilution of the initial supply of hypothetical substance should be particularly rapid in the latter case.

Both these experiments have been performed by Rizet. If each doubling of the mass of protoplasm is called a generation, a very conservative estimate shows that *the modified s character can be preserved through at least 300 generations, in the course of which any substance contained in the modified* s *ascospores with which the experiment was started must have undergone the prodigious dilution of* 2^{300}. The obvious conclusion is that the new character is per-

sistent and therefore cannot be due to the passive transmission of an initial supply of substance.

In other words, the experiments lend no support to the hypothesis of predetermination, and we must therefore try to find another interpretation. Shall we assume that, in the *modified s* spores, the *s* gene (or some other gene) has really undergone a mutation under the influence of *S*?

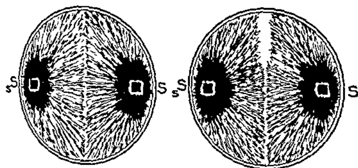


FIG 35 The reversion of s^S to *s* In each of the Petri dishes, one of the confronted strains is *S*, the other s^S. In the Petri dish on the right the occurrence of a reversion is signalled by the presence of a partial barrage (courtesy of Rizet, unpublished).

Before we discuss this, I should like to point out that the emphasis placed on the observed persistence of the *modified s* character should not be taken to mean that, once acquired, this character is irreversible. It is not. Reverse changes, that is reversions from s^S to *s* do occur occasionally. Fig. 35 on the right shows how such a reversion manifests itself when it occurs at some point in a *modified s* mycelium confronted with an *S* strain. If the *modified s* mycelium had remained unchanged, its line of contact with *S* should show no barrage. But here we have a barrage on a part of the line of contact. Apparently, the originally *modified s* culture has formed a sector which has no longer the *modified s* character. Has it reversed to *s* in this sector? This can be ascertained by cutting out small fragments of mycelium just behind the line of contact and by

confronting them with a standard *S* mycelium. Provided this is done as soon as the contact between the two strains has been established, one finds that the fragments taken in the neighbourhood of the barrage form a barrage with *S*, while the fragments taken in the neighbourhood of the simple contact line do not form a barrage with *S*. Indeed, the fragments behave as *s* and s^S mycelia, respectively. However, if the same operation is performed a few days later, a different result is obtained: wherever the sample is taken it forms a barrage with *S*, that is it shows the behaviour of a typical *s* mycelium. In other words, *whenever a reversion to s occurs, it spreads to the rest of the mycelium.* The spread of the change can actually be measured by taking, at 24 hour intervals, fragments of the originally *modified s* mycelium at different distances from the barrage, and testing them by confrontation with *S*. It is then found that the reversion progresses at a speed of about 1 cm. per day.

This most interesting property of the reversion, which is as intriguing as the nature of the initial conversion of *s* into *modified s*, can be demonstrated by inoculating in the same Petri dish three mycelia, respectively *s*, s^S, and *S* (Fig. 36). If none of the strains changes under the influence of the others, a barrage should be observed only between *s* and *S*. This is indeed the case when the three strains are inoculated far apart and at equal distance from each other, so that they all establish contact at the same moment. If, however, *s* and *modified s* are inoculated nearer to each other than to *S*, then there is also a barrage, partial or complete, between the *modified s* and *S* mycelia, which starts from the point of contact of *modified s* with *s*. The smaller the initial distance between *modified s* and *s*, the more extended is this barrage. This clearly shows that the inoculated *modified s* mycelium changes into *s* under the influence of the contact with *s*, that is, under the influence of something emanating from it. It also shows that the reversion is the more complete, the longer

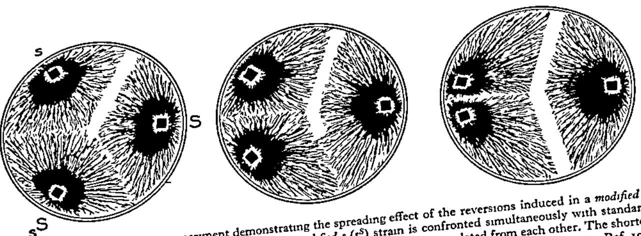


FIG 36 Diagram illustrating an experiment demonstrating the spreading effect of the reversions induced in a *modified s* strain by an *s* mycelium In each of the Petri dishes a *modified s* (s^S) strain is confronted simultaneously with standard *s* and *S* strains The three dishes differ in the distance at which the s^S and *s* are inoculated from each other. The shorter this distance, the longer the barrage formed between s^S and the indicator strain *S* (modified from Rizet, 1952, see Ref. 19).

confronting them with a standard *S* mycelium. Provided this is done as soon as the contact between the two strains has been established, one finds that the fragments taken in the neighbourhood of the barrage form a barrage with *S*, while the fragments taken in the neighbourhood of the simple contact line do not form a barrage with *S*. Indeed, the fragments behave as *s* and s^S mycelia, respectively. However, if the same operation is performed a few days later, a different result is obtained: wherever the sample is taken it forms a barrage with *S*, that is it shows the behaviour of a typical *s* mycelium. In other words, *whenever a reversion to s occurs, it spreads to the rest of the mycelium*. The spread of the change can actually be measured by taking, at 24 hour intervals, fragments of the originally *modified s* mycelium at different distances from the barrage, and testing them by confrontation with *S*. It is then found that the reversion progresses at a speed of about 1 cm. per day.

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fore, if the change from *s* to s^S is a gene mutation, it must be a mutation at the *s* locus. We thus would be in the presence of three alleles: *s*, *S*, and s^S, the last of which is unstable and the other two stable. The instability of s^S would cause this gene to mutate frequently to the stable allele *s*, and never to *S*.

This does not appear as a probable interpretation. Aside from the fact that this would be the first instance ever discovered of an unfailing induction of a mutation of a specific gene, the results of reciprocal crosses between *s* and *modified s* clearly speak against it. Such crosses can be performed by placing a suspension of microconidia of each of the strains on mycelia of the other. The results differ in these two crosses. When *modified s* microconidia are used to fertilize *s* strains, all asci formed contain only *s* spores. In the reciprocal cross the great majority of asci contain only *modified s* spores. The genes contributed by the two parents are the same in the two crosses: therefore, if the characters we are studying depended solely on genes, they should give identical results. However, they do not. The results are what would be expected if the amount of cytoplasm contributed by each of the parents played a decisive part: the microconidia contribute very little, if any, cytoplasm. When they are of the *s* type, they are therefore unable to induce the reversion of the *modified s* ascogone. On the other hand, when they are of the *modified s* type, their character is lost under the influence of the bigger mass of female *s* cytoplasm. Thus, *the results of reciprocal crosses indicate that the factor responsible for the reversion has its seat, if not its origin, in the cytoplasm*, and we may assume as well that *both the change from s to modified s, and the reverse change from* s^s *to s, are modifications of the state of the cytoplasm.*

To what type of cytoplasmic changes they belong, it is at present impossible to say. One can, of course, imagine that one of the mycelial types, either *s* or *S*, owes its character to the presence, the other to the absence of some sort of cytoplasmic particles, and that the two changes,

it has been in contact with *s* before establishing contact with the indicator strain *S*.

About the mechanism of this peculiar spreading effect, which permits the induction of reversions of *modified s* strains to *s* at will by growing them together with *s* mycelia, we know nothing except that it is not due to the penetration of *s* nuclei, followed by the substitution of the latter for the 'native' nuclei of the original s^S mycelium. However, I want you to believe that it is not the desire to impress you with the number of things we do not understand that prompted me to tell you about it: it is rather the desire to show you the difficulties which lie in the way of a precise determination of the frequency of reversions which is important for our discussion. If the reversions were the result of the presumed dilution of a hypothetical substance, *this frequency should increase with the number* of generations, that is with time. I will not go into the details of the method which has permitted Rizet to calculate the relative frequencies of their occurrence in the course of successive generations in spite of the spreading effect of the reversions. The result of the calculation indubitably shows that *the frequency of reversion is constant and independent of time*: reversions are as frequent in a *modified s* strain immediately after its origin in a $s \times S$ cross as 300 generations later, during which interval of time the s^S parts of the mycelia have undergone, as such, an increase of mass of at least 2^{295}. This constancy of the rate of reversion makes them appear much more like true mutations than *like the results of the dilution of* a hypothetical substance.

Shall we then assume that the reversions from *modified s* to *s* which, when they occur, are definitive, are due to true gene mutations which invariably occur under the influence of the contact with *s* mycelia? And, if so, shall we also conclude, as I suggested earlier, that the first change, which invariably converts *s* in the heterozygote *s*/*S* into *modified s*, is also a gene mutation?

The two crosses: $s \times S$ and $s^S \times S$ give identical results. There-

cent of the genetic behaviour of some poorly viable strains of *Podospora*, described by Rizet some years ago.[18]

Rusts. Very similar to the observations of the Mitchells on *Neurospora*, are those of Johnson on rusts.[12]

Space does not allow me to go here into the detailed description of the complicated life-cycle of these parasitic organisms, with their several types of sexual and asexual spores. It will suffice to say that *Puccinia graminis* is a typical heteroecious rust. It parasitizes alternately *Berberis* plants and certain *Gramineae*. On the first of these hosts it propagates in the form of monocaryotic (haploid) mycelia. The dicaryotic phase of the cycle takes place on certain varieties of *Gramineae*, towards which the different 'physiological races' of *Puccinia* manifest a sharply defined host-specificity.

The monocaryotic mycelium growing on *Berberis* eventually gives rise to sexual organs (pycnidia), which contain both flexuous hyphae (♀) and pycnidiospores (♂). Fertilization, however, occurs only between gametes of opposite mating type.

Crosses between 'physiological races', in the majority of cases, give results consistent with the view that pathogenic characters, such as host-range and size of the lesions (sori), are determined by Mendelian genes. However, a few notable exceptions have been discovered. One of them concerns the 'physiological races' 7 and 11 of *P. graminis avenae* which are respectively characterized by type-1 and type-4 sori on the oat variety Sevnothree. When crossed, these races give rise to progenies producing infections typical of the maternal parent.

Similar observations were made on the variety *tritici*, and in this case it was shown that the differences exhibited by the reciprocal hybrids are maintained in further generations (F_2 and F_3).

The observed differences in the results of reciprocal crosses are ascribed by Johnson to cytoplasmic heredity.

Pholiota mutabilis. The persistent influence of the cytoplasm on the growth behaviour of the dicaryophase mycelium of *Pholiota mutabilis* (Hymenomycete) was revealed many years ago by Harder's experiments.[9]

In Hymenomycetes, the dicaryophase mycelium is formed by the fusion of haploid mycelia of complementary mating types, followed by synchronous division of the two nuclei of each cell

from *s* to *modified s*, and the reverse, correspond, one to a 'contamination', the other to a 'decontamination'. But many difficulties appear when one tries to account for all the known facts on the basis of such an hypothesis: the case is very similar to that of the antigenic types of *Paramecium* and could possibly also be accounted for in terms of flux equilibria.

Neurospora crassa. A case of apparently non-chromosomal heredity has recently been described by Mitchell and Mitchell[16] in *Neurospora crassa* which has thus far been considered as a paragon of Mendelian inheritance. The character involved has been designated by the almost self-explanatory term 'poky'. The biochemical basis of the slow growth exhibited by 'poky' strains is as yet insufficiently known, but it is interesting to note that, according to information kindly supplied by the discoverers, 'poky' differs from normal strains of *Neurospora, among other things*, by its content in cytochromes *a*, *b*, and *c*. It will be remembered that these cytochromes are affected by the mutation of yeast discussed earlier (see pp. 23–24).

The life-cycle of *Neurospora crassa* is very similar to that of *Podospora*. The only notable differences to be mentioned are as follows: (*a*) the role of male gamete, played in *Podospora* by microconidia alone, can be assumed in *Neurospora* by plurinuclear conidia and hyphae, as well as by microconidia; (*b*) there are eight uninucleate spores per ascus. This makes the observation of Mendelian segregations particularly easy.

'Poky' is a true breeding character. The spore progeny of a cross between two 'poky' strains of opposite mating type is homogeneous: all ascospores give rise to 'poky' mycelia. Reciprocal crosses between 'poky' and normal, which result from the fertilization of protoperithecia of one mating type by conidia of the opposite mating type, lead to the formation of perithecia of which all the ascospores are of the maternal type with respect to the 'poky' character, while they show normal (Mendelian) segregation of biochemical marker genes. If the protoperithecial (female) parent is 'poky', all ascospores give rise to 'poky' mycelia; if it is normal, all ascospores produce normal mycelia.

The occurrence of the two types of perithecia, homogeneous either for one of the alternative characters or the other, is reminis-

to another in *Paramecium*, and from *s* to *modified s* in *Podospora*.

With these facts in mind we can now return to the problems of cell differentiation and cell heredity in higher forms. In doing so we must remember two things. First, that in our search of the nature and seat of these phenomena in the Metazoan cell, we turned our attention to micro-organisms because we were unable to solve the problem by direct analysis of somatic cells. Our reasoning will be by analogy. We cannot speak, therefore, of what differentiation is due to, only of what it may be due to. Secondly, let us remember that the differentiation of specialized cell types in the course of ontogenetic development was assumed at the outset to be irreversible. I have given you reasons for believing that this is the most plausible assumption. However, a claim of irreversibility is of necessity based on negative evidence. Therefore it cannot be proved; it can only be disproved. Consequently, let us not confine our attention to the equally seemingly irreversible processes revealed by the study of micro-organisms. Any sort of change having a tendency to self-perpetuation is of interest to us. This is so not only because the highly specialized differentiation of lines of somatic cells may, after all, turn out to be reversible under some as yet unknown conditions, but especially because we know that, in the course of ontogenetic development, irreversible differentiation is preceded by stages of labile determination.

Differentiation of cell types, we thought at the outset, must be due to self-perpetuating cytoplasmic changes, but genetic analysis performed on cells of the germ line provided no support to such an hypothesis. However, by studying organisms without isolated germ line we find that *both irreversible and reversible but persistent divergence of cell lines can be achieved through the sole agency of cytoplasmic variation, in the absence of permanent nuclear changes*. Indeed we find more: cytoplasmic variations

and 'clamp' formation. In this process the apical cell of a hypha forms a hook which bends backwards and into which migrates one of the four nuclei. A pair of nuclei of opposite mating type move more towards the apical part of the cell, while the fourth nucleus remains behind. A cell wall is then formed at the base of the hook (or clamp), thus separating it from the cell, and another across the cell, just below the base of the clamp, subdividing the cell into a binucleate apical cell and a basal cell which contains at this moment only one nucleus. This is followed by the penetration of the clamp into the basal cell and the migration of the clamp nucleus into the basal cell. This process is started over in the new apical cell. Eventually a dicaryotic mycelium is produced.

Harder isolated haploid mycelia of *Pholiota mutabilis* of complementary mating types, which differed in origin and in growth pattern. Fusion of the haploid mycelia of two strains, A and B, was observed under the microscope. The clamp and the apical cell were destroyed by cutting them with a needle before the clamp connexion was established, and the basal cell was separated from the mycelium. Thus, cells were obtained which contained mixtures of A and B cytoplasm and only one (B) nucleus, recognized by its mating type.

Like the mycelia of the ordinary $A \times B$ hybrids, the haploid mycelia produced by these 'merogonic hybrids' showed growth characteristics intermediate between those of the pure strains A and B. These characteristics were maintained over prolonged periods of vegetative reproduction.

2. GENERAL DISCUSSION

I have now presented enough facts to enable us to answer the question we started with (Introduction): can divergence of cell lines be due to cytoplasmic changes? Indeed, we find that it often is. The cytoplasmic differences, we found, belong to two different classes. Some changes of cell type appear to be irreversible: such are the changes from normal to vegetative littles in yeast and from killer to sensitive in *Paramecium*. Others are persistent, but reversible: such are the changes from one antigenic type

to another in *Paramecium*, and from *s* to *modified s* in *Podospora*.

With these facts in mind we can now return to the problems of cell differentiation and cell heredity in higher forms. In doing so we must remember two things. First, that in our search of the nature and seat of these phenomena in the Metazoan cell, we turned our attention to micro-organisms because we were unable to solve the problem by direct analysis of somatic cells. Our reasoning will be by analogy. We cannot speak, therefore, of what differentiation is due to, only of what it may be due to. Secondly, let us remember that the differentiation of specialized cell types in the course of ontogenetic development was assumed at the outset to be irreversible. I have given you reasons for believing that this is the most plausible assumption. However, a claim of irreversibility is of necessity based on negative evidence. Therefore it cannot be proved; it can only be disproved. Consequently, let us not confine our attention to the equally seemingly irreversible processes revealed by the study of micro-organisms. Any sort of change having a tendency to self-perpetuation is of interest to us. This is so not only because the highly specialized differentiation of lines of somatic cells may, after all, turn out to be reversible under some as yet unknown conditions, but especially because we know that, in the course of ontogenetic development, irreversible differentiation is preceded by stages of labile determination.

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of interest to us can be achieved by more than one mechanism; irreversible changes have been shown to be due to the segregation of self-reproducing cytoplasmic elements; reversible changes, on the contrary, are not due to qualitative changes of the intrinsic composition of cells: they may or may not (and probably do not) involve variation of self-reproducing particles (see Note 1). Moreover, we find that cytoplasmic variations of both persistent and irreversible character can be initiated in several ways. The non-living environment can induce changes of concentration of kappa particles and of antigenic type in Paramecia, and the loss of cytoplasmic particles in yeasts. The contact with living environment (*s* strains) induces the change from s^S to *s* in *Podospora*. The reverse cytoplasmic change in this organism is apparently due to the interaction of certain nuclear genes. Lastly, we find that nucleus and cytoplasm affect each other's activity. The cytoplasmic particles of yeasts are activated by a nuclear gene. In turn, in Paramecia definite cytoplasmic states permit the expression of definite nuclear genes.

Here is a set of facts that ought to help explain development.

Considering that embryonic development results in a restriction in different cell lineages of the manifold potentialities originally carried by the egg,* we may picture the process of differentiation as consisting, for example, in the segregation, or sorting out, of an initially mixed population of cytoplasmic particles. Or we may suppose that the egg, to begin with, contains a mixed population of inactive particles, and that development consists in the activation by nuclear genes of different sorts of particles in different cell lineages (see Notes 2 and 3). Many other schemes of this sort can be set up: the study of micro-organisms has supplied us with several

* This statement is to be taken as a first approximation only, for the work of Spemann[33] has shown that development consists in simultaneous restriction and widening of the potentialities of the egg.

models of differentiation based on purely cytoplasmic mechanisms or on nucleo-cytoplasmic interactions (see Note 4). It will be the task of the embryologist to choose among them.

Let us not delude ourselves, however, and think that we have supplied him with the key to his problems: the embryologist is not in sight of the end of his task; and neither are we. *While any one of the cytoplasmic mechanisms described could be an instrument of somatic differentiation, none of them can be its primary factor.*

Development is an orderly process: it follows a 'plan' which dictates when and where the instruments of differentiation come into action. Experimental embryology has taught us that a more or less rough outline of this 'plan' is engraved already in the cytoplasm of the undivided egg. Sometimes it is indicated by the visible distribution of cytoplasmic materials, sometimes it can be revealed only by experiment. But at this stage the 'ground plan' is neither complete, nor necessarily definitive: it will be gradually refined and fixed in the course of subsequent development.

The primary cause of differentiation thus resides in the initial anisotropy of the egg, expressed by its polarity and symmetry. At first sight this initial anisotropy could be set up by rather trivial external factors. However, it is sufficient to compare the 'ground plans' of the eggs of representatives of different groups of vertebrates (Fishes, Amphibians, Reptiles, &c., Fig. 37) to discover a most striking similarity of plan. Inasmuch as we admit that the different homologies in these groups of organisms reflect a common descent, we are left with no doubt that *the fundamental anisotropy of the egg cytoplasm itself has a genetic basis.*

It must be recalled now, that the 'ground plan' is not always definitively fixed in the undivided egg: the blastomeres resulting from its division often remain capable, when isolated, of developing into complete organisms,

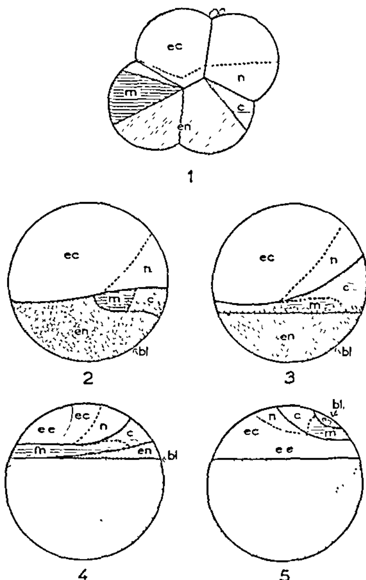


FIG. 37. Dalcq and Pasteels's topographical chart of organ specific areas in the eggs of *Amphioxus* type (1), of a *Cyclostome* (2), an *Anuran* (3), a *Teleost* (4) and a *Sauropside* (5). bl, blastopore; c, presumptive notochord; ec, presumptive ectoderm, ee, presumptive extraembryonic ectoderm; en, presumptive entoderm; m, presumptive mesoderm; n, presumptive nervous system (after Fauré-Fremiet, 1943, see Ref. 4).

strictly similar to those formed by the whole egg. Each of these parts of the egg therefore carries within it the same 'ground plan' as the egg as a whole. *The anisotropy of the egg, sometimes revealed by the distribution of cytoplasmic materials, is therefore only a reflection of another,*

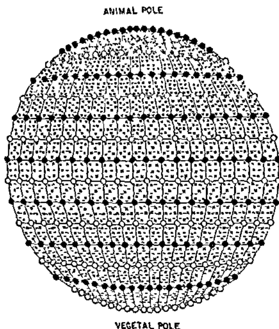


FIG. 38 Harrison's diagram of an ovum, illustrating the two factors in polarity There is a protein lattice made up of black and white circles oriented towards the two poles, and constituting the 'polarity of direction'. In the interstices of the lattice are particles of two kinds designated by × and −, one of which is more concentrated at the animal pole and the other at the vegetative pole, and indicating the 'polarity of stratification', in accordance with the Runnström-Hörstadius theory of the sea urchin egg (from Harrison, 1945, see Ref. 9)

more fundamental and deeper asymmetry, presumably seated on the molecular level[4, 5, 7, 9, 14] (see Note 5).

A model of the molecular structure of the egg cytoplasm, due to R. G. Harrison,[9] and viewed as a lattice of oriented bipolar protein molecules, in the meshes of which

are contained the various cytoplasmic inclusions, is shown in Fig. 38. This structure possesses a 'primary polarity', due to the orientation of the dipole molecules of the lattice, and a 'polarity of stratification' due to the distribution of secondary materials. It is easy to imagine how such a system, anisotropic from the start, can, under the influence of stimuli emanating from the nucleus, from the environment, or from a cytoplasmic inclusion, evolve asymmetries of a higher order.

Harrison's model may give us only a much simplified picture of reality, but, if we accept it at all, *the fundamental problem of genetics in relation to development becomes that of the origin of the specific molecular pattern of the cytoplasm, which confers to the egg its vectorial properties* (see Note 6).

However, even the solution of this problem will not be the end of the journey. The fact that each of the parts of the egg is capable of developing into a complete organism, and yet does not do so when left in its natural position, proves that the developing germ, the embryo, is an integrated unit.[4, 9, 27] Indeed, it has the properties of a 'supra-cellular continuum'.[27] The ground plan, potentially contained in each of the cells, is now superseded by *that of the embryo as a whole, of which the individual* cells are now only the subordinate, executive agents. Cell boundaries appear to be no obstacle to the all-pervading integrative forces, the nature of which is the key to biological organization (see Note 7).

This reference to the properties of the organism as a whole contains within it the limitation of the cell-theory in its original form. Morphogenesis, we know now, is not dependent on the subdivision of the organism into cellular units: it can be achieved with or without such subdivision. Strikingly similar forms can be assumed by unicellular and multicellular organisms. The emphasis is here placed therefore not on the subdivision of the organism into cells, but on the integrated character of the organism, whether unicellular or multicellular. The cell is, however, the

lowest biological unit endowed with the integrating properties of an organism.

With this in mind, let us now return to cell heredity which, we thought at the outset, must have its seat in the cytoplasm. Indeed, our study gives support to this view. We have seen that, in *irreversible* divergence of cell types, cell heredity is the consequence of the genetic continuity of sometimes visible cytoplasmic elements. What guarantees persistence of the *reversible* changes of cell type is impossible to say at present, but it is clear that, whatever its mechanism, it also has its seat in the cytoplasm.

The cytoplasm thus appears to possess a degree of autonomy high enough to make it no longer tenable to limit its significance to a subordinate role in the life of the cell.

However, high as the degree of autonomy of any cell element may be, it is limited: this holds for the nucleus as well as for the cytoplasm. The particulate genetic elements of the cytoplasm of the yeast cells and of Paramecia have been found to be dependent on the nucleus in their reproduction or activity. Similarly, the alternative cytoplasmic states revealed by the study of antigenic types of Paramecia depend on the nucleus, which defines their range of possible variations and the specificity of the antigens produced. In turn the cytoplasmic states of Paramecia determine which of the nuclear genes comes to expression. Again, in yeast, the nuclear gene required for the production of respiratory enzymes cannot express itself unless a cytoplasmic particle is also present. To ascribe the primary role in this process to the nuclear component of the cell, rather than to the cytoplasmic one, has no more meaning than the reverse. *The cell is an integrated unit which can be broken down only for purposes of analysis.*

It may be argued, of course, that, strictly speaking, we have shown only that the cell is a unit of function, and that it is still possible that the property of identical

reproduction is the exclusive attribute of subcellular elements, whether nuclear or cytoplasmic. While there is no doubt in my mind that this unique property of living matter has its seat in the specific molecular organization of subcellular units (see Note 8), I do not think that it is possible to draw a line between the two aspects of the cell: *cell-reproduction is inseparable from cell-function* (see Note 9).

Here again we can reason only by analogy. Virus particles,[10] regarded as close analogues of the normal self-reproducing elements of the cell, are apparently barely more than small groups of genes. Viruses seem to possess no enzymatic activity with which to obtain the energy required for their autosynthesis. In order to multiply, they mobilize to their advantage the highly co-ordinated metabolic activities of the host cell. Their autonomy in reproduction is therefore limited. In fact, it is so dependent on the metabolism of the cell that one may as well speak of the cell reproducing the virus, as of the virus reproducing itself. The self-reproducing character of the viruses is the result of the presumably last act of their synthesis, the act in which a pre-existing element, by supplying a molecular pattern to be copied, imposes on the products of the integrated cell metabolism its own configuration and specificity.

All that was said about viruses, presumably holds also for the normal subcellular elements, including the nuclear genes.

We may therefore speak of autoreproduction of the integrated unit which is the cell, but it would be more correct not to speak of autoreproducing particles: in view of their unique role in the process of identical perpetuation, I would rather speak of them as *conservative units* of the cell.

Let me supplement these considerations by a quotation from a recent article by Sonneborn: 'If the nucleus were in complete and exclusive control of heredity, then it would have to be concluded that nuclei, isolated under

conditions that permit their multiplication, would be capable of reconstituting cells of the kind from which they were taken. If this did not happen, then it would have to be concluded that the cell, including the cytoplasm, somehow serves as a necessary model for the formation of new cellular material in essentially the same sense as the genes are necessary models for the formation of new genes. The unicellular colorless flagellate *Chilomonas* can be cultivated on a few inorganic salts and acetic acid. Would any biologist go so far as to believe that a successful culture of *Chilomonas* nuclei, provided with such a diet—or for that matter with as complex a diet as one wishes—could reconstitute a *Chilomonas* cell, or any cell at all? Yet if the cytoplasm is entirely the result of gene activity, if it can all be made *de novo*, when food is provided, no model of pre-existing cytoplasm should be needed for its new function.

'Perhaps it will be objected that there are some self-duplicating cytoplasmic elements which the nucleus cannot make. Then suppose these too can be cultivated *in vitro*. Is anyone willing to believe that, if all such self-duplicating components of the cell were thrown together in a test tube in the proper proportions with adequate food for their multiplication, a *Chilomonas* cell or any cell at all would result? Although the whole picture is admittedly imaginary, it makes the nature of the problem sharp and clear. If cells cannot be reconstituted in the way suggested, then it seems to me we are forced to admit that the molecular and particulate arrangement of the cellular materials, their organization into a working system, is itself a part of the genetic system of the cell.'[22]*

After a century of amazing progress in the analysis of the cell and its genetic structure, we thus return to the notion of the cell as the ultimate unit of life, lost in the course of our advances. This loss was the inevitable logical consequence of the analytical methods employed.

* Cf Pontecorvo's paper 'Genetical analysis of cell organization' given at the 6th Symposium of the Soc. Exp. Biol. 1951.

The present knowledge of the biochemical constitution of the cell was achieved largely by the use of destructive methods. Trained in the tradition of the theory of solutions, many a biochemist tends, even today, to regard the cell as a 'bag of enzymes'. However, everyone realizes now that the biochemical processes studied *in vitro* may have only a remote resemblance to the events actually occurring in the living cell.

It is less obvious that the method of genetics, although it involves no 'bloodshed', is as analytical in its essence. Indeed, the 'resolving power' of this method is amazing. It provides us with a picture of the cell's nuclear constitution with unequalled 'definition'. But, so long as the basis of genetics is the study of differences, it cannot be expected to give us an undistorted picture of the cell as a whole. The integrated character of the cell, which is its fundamental property, is bound to escape our notice most of the time.

These statements are not intended to imply that the current analytical trends and methods are bound to be fruitless. All I want to convey to you is that equal emphasis should be placed on the study of the processes of cell integration.

Neither should my opinions be taken as a sign of defeatism, for they are based on the belief that ultimately cell integration will find its explanation in terms of knowable, if not known, molecular structures and forces.

The journey to this goal will be long and hard indeed, but at its end lies a great promise: the insight into the mystery of the individuality of the living.

NOTES TO DISCUSSION

1. Wright, who was among the first to speak of steady states in connexion with the genetic problem of differentiation, remarks that 'The idea of differentiation of the cell as a self-regulatory system

as a whole and that of stabilization of a particular mode of differentiation by means of the elaboration of self-duplicating entities within the cell are not incompatible'.[29]

It must be added that, putting aside cellular changes involving visible cytoplasmic elements, the distinction between changes based on transitions from one stable state to another, and those due to shifts in composition of populations of plasmagenes, is *a priori* very difficult. Steady states should be reversible, but so should be shifts in populations of plasmagenes.

2. Wright[23] suggests that plasmagenes may be transmitted by the germ line of higher organisms in an inactive form, lacking the prosthetic groups, and that, in the course of development, they are irreversibly activated for different functions by combining with such groups emanating from the nucleus. Because of differences in local conditions, the plasmagenes 'activated' in this way are of different sorts in different somatic lines. All of them are capable of multiplying in an independent fashion in the activated form and this results in cell heredity.

It may be pointed out that Wright's hypothesis that plasmagenes may be transmitted in an inactive form finds a confirmation in the fact that, in the 'segregational mutants' of yeast, non-functional cytoplasmic particles remain capable of multiplication. Furthermore, it has been shown above that these particles are indeed activated by something emanating from the nucleus. However, the requirements of Wright's hypothesis are not entirely fulfilled in this case, because the activity of the cytoplasmic particles ceases as soon as they are introduced into a cell carrying the recessive gene *r*.

3. Extensive studies of the biochemical changes occurring during ontogenesis have led Brachet to rather similar views, as shown by the following quotation: '. . . l'œuf vierge contiendrait surtout de très petits granules, extrêmement riches en acide ribonucléique, à mesure que le développement progresserait, ces granules se compliqueraient progressivement et deviendraient quantitativement, puis qualitativement différents. Ces granules, qui contrôleraient l'organogenèse, donneraient ensuite naissance à des protéines spécifiques lors de la différenciation histologique des organes. Le fait que ces granules, à en juger par nos études sur l'acide ribonucléique et celles de Barth sur la respiration des hybrides létaux, ne semblent pas être à même de se synthétiser

lorsque la constitution génétique des noyaux est anormale, rend probable l'idée que leur multiplication se trouve sous le contrôle des gènes. On en arriverait ainsi à l'hypothèse suivante: les remaniements cytoplasmiques qui suivent la fécondation rendraient le cytoplasme hétérogène; ces différences cytoplasmiques retentiraient, à la fin de la segmentation, sur l'activité de certains gènes dans les divers territoires. C'est sous l'influence des gènes que s'opèrerait la synthèse des granules qui, à leur tour, seraient les agents de la synthèse des protéines.'[2] (Cf. also Lehmann[13].)

4. Such cytoplasmic changes may of course, in turn, affect the nuclei. As stated in the Introduction, the functional equivalence of the nuclei of different lines of differentiated somatic cells has not been proved. Goldschmidt has long ago suggested that development consists in the orderly activation of different sets of genes in the different cell lineages. The experiments of Beale quoted earlier (pp. 69–75) show such a cytoplasmic effect on genic activity. It may be added that, as Sonneborn[21] has pointed out, in *Paramecium* the production by the fertilization nucleus of two different sorts of nuclei (macro- and micro-nuclei) 'shows in an unambiguous way what is familiar to protozoologists, but appreciated by relatively few other biologists, namely, that nuclei themselves may become differentiated during development, in the absence of reduction divisions and without genic segregations'.

5. The same conclusion is reached when one considers the fact that, in the regeneration and asexual reproduction of many lower forms, the development of a complete new organism can be started from a few cells which have arisen under conditions very different from those which have presided at the formation of the egg itself. These remote descendants of the egg cell then also carry 'the plan' within them.

6. In the organization of the egg the cortical structures are of particular importance: it is the cortex which appears to be directly responsible for the vectorial properties of the egg.[3, 24, 27]

Similarly, in unicellular organisms the structure of the cortex has been shown to be associated with the organization of the individuals and their morphogenetic changes.[17] In Ciliates numerous observations and experiments show that the infraciliature, that is the orderly system of kinetosomes and kinetosome-connecting fibres

(*kinetodesma*) is directly connected with all morphogenetic processes.[6,15,21,25] The division of a simple ciliate is preceded by the reorganization of this network of the infraciliature, which permits one to observe, long before nuclear division and cytoplasmic cleavage, the substitution of two systems for the previous single one, so that a simple constriction of the cytoplasmic body can separate two complete individuals.[6]

The distribution of the complex system of kinetosomes no doubt reflects the more profound underlying molecular pattern of the cortical cytoplasm.[5,6,15] Thus, morphogenesis in Ciliates and ontogenetic development of Metazoans alike confront us with the problem of the nature and origin of the specific molecular pattern of the cytoplasm.

7 The fundamental unity of the cell has prompted Baitsell to write: 'May it not be possible that the cell is essentially a protoplasmic crystal in which an almost infinite number of protein molecules, beginning with the genes in the chromosomes, are associated in a definite ultramicroscopic pattern characteristic of the particular type of cell? In such a condition, just as Langmuir early recognized, individual protein molecules are not present, but all are united to form the perfect unit, the complete crystalline pattern of a specific type of protoplasm, the terms molecule, crystal and cell becoming synonymous.'[1]

Similar speculations have been advanced by several authors on the basis of consideration of the integrated character of more evolved organisms and of their development. From his study of the development of an insect, Wigglesworth, for example, concludes: 'The prime mover in morphogenesis in the insect is the epidermis. The executive units in the process are the individual cells; but the functions which fall to the lot of a given cell are determined by the non-cellular continuum which is the essential organism.'[27]

Elsewhere, Wigglesworth remarks: 'The essential organism is something apart from the cells which support it. It exists before the cells dispose themselves and define its form. The cells and their nuclei, as the vehicles of the genes, play a great part in controlling the details of the form the organism will take; but the framework which marks the main outlines of *that form*, which says that the organism shall be a vertebrate, an amphibian, a frog, or an insect, a dipteron, a *Drosophila*, which defines the head and the tail, the

main regions of the body and the limbs—this framework exists before the cells.'[26]

8. Sonneborn[22] has pointed out: 'The cytoplasm may provide a particular self-perpetuating molecular pattern. This could be true both for the surface of self-duplicating particulates, such as plastids and mitochondria, and for the fibrous ground substance of the cytoplasm. There are, it seems to me, reasons for suspecting that *the molecular organization of the cytoplasm may be a hereditary* property of the cytoplasm, comparable to the hereditary arrangement of the genes in the chromosomes. The rapid and efficient operation of enzyme systems with many enzymes participating in a regular sequence, seems to require a precision of *localization on enzyme-bearing particles such as mitochondria*; and this arrangement is most easily conceived as a consequence of the surface pattern on which the enzymes are adsorbed. As the mitochondria are probably self-duplicating, the pattern too may be perpetuated.'

9. The reader might be interested in the following quotation from Hopkins:[11] 'You will at once urge, and, of course, rightly, that the attributes of a living system go far beyond its power of maintaining in various environmental circumstances a dynamic individuality. A cell has a history; its structure is inherited, it grows, divides, and, as in the embryo of higher animals, the products of division differentiate on complex lines. Living cells, moreover, transmit all that is involved in their complex heredity. I am far from maintaining that these fundamental properties may not depend upon organisation at levels above any chemical level; to understand them may even call for different methods of thought; I do not pretend to know. But, if there be a hierarchy of levels, we must recognize each one, and the physical and chemical level which, I would again say, may be the level of self-maintenance, must always have a place in any ultimate complete description.'

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ADDENDUM

'Thus there can be no doubt that there are qualities of the cytoplasm that are autonomous. The astonishing thing is that cytoplasmic heredity plays such a small role'
(S. WRIGHT, The physiology of the gene. *Physiol Rev.* 1941, 21, 487–527)

ON THE SCARCITY OF EVIDENCE OF CYTOPLASMIC HEREDITY, AND ON THE AMBIGUITY OF THE CONCEPT OF AUTOREPRODUCING UNITS

IN his notably lucid discussion of the genetic problem posed by cellular differentiation, S. Wright states: 'The usual and most probable view is that cellular differentiation is cytoplasmic and must therefore persist and be transmitted to daughter cells by cytoplasmic heredity. The chief objection is that it ascribes enormous importance in cell lineages to a process which is only rarely responsible for differences between germ cells, at least within a species.'[14]

Although several new instances of cytoplasmic heredity have come to light since the publication of Wright's article, the objection is still valid. Whatever credit is given to the evidence presented in my lectures, it remains a fact that the well-authenticated cases of cytoplasmic inheritance are very rare, and that Mendelian heredity is, on the contrary, of universal occurrence among all organisms, whether animals, plants, or micro-organisms, subjected to genetic analysis.

Since, in the foregoing, I have placed a great deal of emphasis on the occurrence of cytoplasmic heredity in micro-organisms and on the theoretical necessity of assuming that differentiation in higher forms involves similar cytoplasmic phenomena, a discussion of the possible reasons for the apparent rarity of cytoplasmic heredity seems to be pertinent at this point.

One possible reason is suggested by Wright himself: 'It may be . . . that the more or less complete early isolation of the germ line has come about in evolution to maintain a line of cells with plasmagenes lacking in prosthetic groups and hence in specialized activity but capable in somatic cells of combining with such groups emanating from the nucleus to form molecules that multiply thereafter as plasmagenes of a more specialized sort.'[14]

The absence of an isolated germ line may indeed be the reason why examples of cytoplasmic heredity are apparently relatively more abundant in micro-organisms than in higher forms.

As pointed out by Sonneborn,[13] the relatively rare detection of cytoplasmic heredity may be due, at least in part, to the methods employed: 'In the first place, there is a simple, familiar and highly successful method—the Mendelian method—for studying genic inheritance; no method comparable in simplicity, power, and reliability is available for the study of cytoplasmic inheritance. This results in considerable selection in the examples of heredity studied and reported in the literature: the simpler cases that yield readily to a familiar methodology are preferentially attacked and reported. Complex or less standard cases tend to be put aside or interpreted formally in terms of accepted theory. Secondly, genetic methods are designed chiefly for analysis of differences between individuals that can interbreed; hence, properties common to all individuals of an interbreeding group remain largely unanalysable by the ordinary methods of genetics except under unusually favorable conditions.'

This quotation contains several independent and important suggestions which deserve particular consideration.

The first I should like to discuss is whether experiments conducted by means of the Mendelian method should be expected to detect cytoplasmic inheritance when it occurs. The question is usually answered in the affirmative, at least implicitly, for it is generally assumed that cytoplasmic

heredity should manifest itself by dissimilar results of reciprocal crosses. This expectation is based on the fact that the egg-cell contains a great amount of cytoplasm, while the spermatozoon carries very little of it. This fact may, however, be very misleading. Cytoplasmic elements which can be thought, on the grounds presented in these lectures, to be endowed with genetic continuity, are of microscopic or sub-microscopic size. There is undoubtedly enough room for such cytoplasmic bodies in the cytoplasmic layer which encloses the nuclear component of the spermatozoon and, especially, in its middle-piece. In fact, it is well known that mitochondria are almost invariably carried in the middle-piece and thus introduced into the zygote which, consequently, contains mitochondria of both paternal and maternal origin. It is usually assumed also that, contrary to the nuclear elements, the cytoplasmic ones are distributed at random at cell division and should therefore be subject to frequent loss. Actually, in many cases a rather even distribution of mitochondria at mitosis has been observed, and it is entirely possible that it is guaranteed in most cells by some as yet unknown regulatory mechanism. Moving pictures of dividing cells certainly do not give one the impression that cytoplasmic elements such as mitochondria are distributed at random between the two sister cells. Yet it is possible that the high frequency of loss of the cytoplasmic particles (probably mitochondria) in yeast is correlated with their reproduction by budding —a mode of reproduction which makes the presumed sheltering mechanism more apt to fail.

For these reasons, the emergence in a higher organism of the equivalent of a 'vegetative mutant' of yeast would be an extremely unlikely event. Furthermore, an individual of this sort would be regarded as a phenotypic variant, for its character would most certainly disappear on crossing.

While a new mutant emerging as a result of a cyto-

plasmic loss-mutation is thus most likely to remain undetected, other reasons may be suggested for the non-detection of established lines of such mutants. If it is assumed that all cytoplasmic particles, like those of yeasts, depend on nuclear genes for their function, and if it is further assumed that the presence of inactive particles confers on the cell carrying them no selective advantage, it is clear that mutation of the genes in question will almost inevitably be followed by the loss of the corresponding cytoplasmic particles.* The cross of such 'dual mutants' with 'normal' will, of course, give a purely Mendelian result and the loss of the cytoplasmic factor will remain undetected.

Sonneborn's second suggestion implies that differences between non-interbreeding groups may be due to cytoplasmic differences more frequently than those occurring between individuals which can be crossed. This is a plausible view, and I am prepared to accept it as well as what, in my opinion, are its two logical consequences: that, on the one hand, 'macro-evolution' has had recourse to cytoplasmic as well as genic mutations, and that, on the other hand, cytoplasmic heredity is particularly concerned *with fundamental cellular functions. If the latter were true,* cytoplasmic mutations would be most of the time incompatible with survival and therefore escape detection. In some instances the loss of cytoplasmic elements endowed with genetic continuity was in fact shown to be incompatible with survival (see above pp. 10–13). Most illuminating in this respect is the example of the yeast cell: here *a fundamental function, respiration,* can be abolished with almost complete impunity only because the organism in question possesses a remarkably efficient alternative

* The presence of physiologically inactive particles presumably confers no selective advantage on the cell which carries them, for cultures of 'segregational littles' are rather rapidly converted into cultures of 'dual mutants'. Stocks of segregational littles are, however, readily recovered by crossing the dual mutants with normal yeast and isolating the ascospores carrying the recessive *r*.

energy-yielding metabolic pathway. It is to this circumstance, hardly realized with similar perfection in many other sexually breeding organisms, that we owe the possibility of its detailed study. The cytoplasmic control of fundamental functions may escape detection precisely for this reason.

The emphasis placed in the above statements on the term 'fundamental' will probably be strongly objected to by many a reader as reminiscent of the old distinction between 'general' and 'special' heredity (cf. Brachet[2]) and the controversies as to whether gene mutations concern both 'superficial' and 'fundamental' characteristics, rather than the former alone. I think that the question is today in need of serious reconsideration, and that it should not be answered by metaphors or by the usual counter-questions, as in the following quotation from a well-known book on the genetical mechanisms of evolution:

'It has been contended, for instance, that mutations [meaning gene mutations] involve only 'superficial' characteristics, leaving the 'fundamental' ones unaffected. Those making such assertions have wisely refrained from revealing their criteria for the discrimination between superficial and fundamental traits.

'The presence of one pair of wings and one pair of balancers, as opposed to two pairs of wings, is one of the most striking distinguishing marks of the order of flies (*Diptera*). One may ask then, is the appearance of a four-winged *Drosophila* a fundamental or a superficial change? Is a mutation which diverts the embryonic development to a wrong course and thus causes death fundamental or superficial? Those who would like to see a mutant fly without an alimentary canal, or with the location of the heart and the nerve chain exchanged, overlook the fact that such a mutant could not survive and hence could never be detected.'[3]

If a clear insight into the problem is to be gained, issues must not be confused, in the first place. The term 'funda-

mental', when applied to the species, to the individual organism, or to the cell, has obviously different meanings. What is fundamental to the cell is of necessity fundamental to the species also, but the proposition cannot be inverted. In a Metazoan a change resulting in a selective advantage of a fraction of a per cent. is of fundamental importance to the species, but is most certainly not a fundamental cellular change. Secondly, we must keep in mind some basic notions of physiological genetics and embryology. If this is done, reasonable answers to the quoted questions may be found.

To the first question the answer appears to be that, whatever the value of the two-winged condition to the *Drosophila* species, the non-fundamental character of this trait to the individual and its constituent cells is certified by the very fact of the survival of four-winged flies. The two-winged condition is obviously more important to the systematist than to the fly, but even to the former it is not as fundamental as the author of the quoted paragraph implies: I am sure that if he ever collected an insect in the field which resembled *Drosophila* in every way except in having four wings, he would still not hesitate to classify it as *Drosophila*.

The answer to the second question is, I think, as follows: 'A mutation which diverts embryonic development to a wrong course and thus causes death' may or may not be fundamental. Lethality of embryos often is due to the breakdown of the correlation which assures the harmonious development of the parts of an embryo. Such a breakdown is the amplified effect of changes which in themselves, that is on the cellular level, are insignificant or confined to particular cell-lines.

The fact that a 'fly without an alimentary canal, or with the location of the heart and the nerve chain exchanged' could not survive is apparently more readily remembered than the possibility that this very fact is the cause of the apparent rarity of cytoplasmic heredity.

I should like to conclude these remarks by a rapid consideration of two constantly recurrent arguments in favour of the predominant role of the nucleus.

It is frequently stated in a rather sweeping fashion that all the major events in the synthetic processes of the cell connected with its growth and multiplication are controlled by nuclear genes. The first point I want to make is that, contrary to this generalization, no convincing proof is available for the direct nuclear control of the structure of the most important cytoplasmic components—the proteins—and their specificity. The most frequently quoted example is that of the Mendelian inheritance of antigenic specificity. In all cases subjected to both genetic and chemical analysis this specificity has, however, in so far as I know, invariably proved to be due to the haptenic component (polysaccharide) of the conjugated proteins. With the rise of genetics of micro-organisms, numerous examples of biochemically deficient mutants have been accumulated which usually have been interpreted in terms of modifications of enzyme specificity. A recent discussion at the Cold Spring Harbor Symposium[7] has brought to light a number of possible alternative interpretations. While there is thus no decisive proof for the nuclear control of enzyme specificity, and no proof that the nuclear genes are directly involved in the immediate synthetic steps resulting in the formation of molecules of the size of proteins, Slonimski[12] has produced what I take as a rather convincing demonstration that the respiratory deficiency of the 'vegetative littles' of yeast is an effect of the mutation on the protein moiety, rather than on the prosthetic groups of the cytochromes. It appears to me, therefore, entirely possible that control of the basic structure of cytoplasmic proteins is a function of cytoplasmic elements.* This possibility has been earlier

* This view is consistent with Haurowitz's[8] hypothesis as to the mechanism of protein synthesis. The essence of this hypothesis is that proteins are formed by 'copying' protein-templates. In the first, synthetic phase of the process, a two-dimensional protein is copied, by a process

reckoned with by Wright, who, in discussing it, adds: 'There is the difficulty here that if the main constituent of the cytoplasm is autonomous one would expect more abundant evidence of cytoplasmic heredity than is found, particularly in connection with specificity.... It is possible that specificity effects due to haptens whose presence is controlled by particlar nuclear genes . . . overshadow specificity due to protein plasmagenes.'[14]

The other reason for ascribing to the nuclear genes an exclusive position in the cell is based on an evolutionary consideration. As Muller[10] has pointed out, the nuclear genes possess 'the attribute most essential . . . as the basis of evolution, that is, the ability to change in such a way as to be able to reproduce themselves in their new form'[11] (covariant duplication). However, as stated earlier, the autonomy of any cell element in reproduction can only be relative: because the autoreproduction of the most specialized structure may hinge on the presence of some very trivial substances, the limits of an autoreproducing system are impossible to define. It is for this reason that, in the definition of autoreproducing elements, an exclusive importance is usually ascribed to the criterion of co-variant reduplication. The point I want to make is that this introduces an ambiguity into the concept of autoreproduction.

Neither the logical nor the material 'isolation' of an element endowed with genetic continuity permits one to define the limits of an autoreproducing system. The fact, for example, that specific nucleic acids of *Pneumococcus*, which direct their own specific (co-variant) reproduction

essentially similar to crystallization, in which 'the role of the nucleic acid is, apparently, to maintain the template protein film in the expanded state'. In a second phase the two-dimensional copy is folded in a complementary fashion to a secondary globular template

The role of cytoplasmic particles (mitochondria, 'biosomes' of Lehmann, 'microsomes' *of Claude, Brachet, and Chantrenne*) in protein synthesis is also suggested by the work of Claude,[2] Brachet,[3] Chantrenne,[4] and Lehmann.[5]

as well as the synthesis of specific polysaccharides, can be isolated in pure form,[1] does not by any means prove that the stuff contained in the test-tube represents the autoreproducing system. Although 'transformations' performed by means of this substance ('transforming principle') are formally equivalent to the transfer of genes, there is not a shadow of doubt in my mind that in the bacterial cell the nucleic acids do not multiply until they are associated with other (possibly protein) substances. Their role in 'transformations' merely *signals* the existence of an autoreproducing system which, apart from the nucleic acid, may comprise elements, the variations of which may or may not be reproduced, but which are, nevertheless, autonomous in the sense that their loss is equally irreparable. The criterion of 'irreparable loss' is, therefore, in itself sufficient for the claim that the element possessing this property represents a part of an autoreproducing system. This is the more so because, on the one hand, in most cases, 'loss' cannot be distinguished from irreversible inactivation with respect to the heterocatalytic function of the element in question, and because, on the other hand, apparent absence of co-variant reproduction may be the result of the incompatibility of the variation of an element with the survival of the cell carrying this element in the modified form. But even assuming that some indispensable elements of autoreproducing systems lack the capacity of mutation and co-variant reduplication, there is no justification for brushing them aside as merely 'primers' of autocatalytic systems, that is, as elements which have nothing to do with hereditary determinants *sensu stricto* (cf. Muller[11]). The conclusion that we are dealing in such a case with rather trivial autocatalysis is precluded by the very fact of the irreparable character of *their* loss. The nuclear genes themselves may very well comprise, in addition to the constituents capable of reproducible variation, indispensable elements of this second sort, and the evolutionary role of

the genes may therefore be dependent upon the integrity of these elements.

The value of autonomous cytoplasmic elements for the evolutionary process is an independent problem. There is no doubt that the property of co-variant reduplication confers on its carriers a special biological role. Such is the case of nuclear genes. The question of whether autonomous cytoplasmic components are also endowed with this property and, consequently, are also capable of 'following an unlimited evolutionary course'[11] will be answered by the establishment of the site of synthesis of cytoplasmic proteins and of the cytoplasmic or nuclear origin of their specificity.

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